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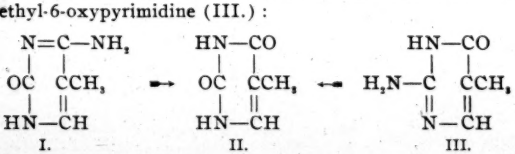
CXVI.—RESEARCHES ON PYRIMIDINE DERIVATIVES: 5-METHYLCYTOSINE.

[FIFTH PAPER.]

BY HENRY L. WHEELER AND TREAT B. JOHNSON.

In a previous paper we[1] showed that cytosine gives uracil on heating with acids. If uracil is to be reckoned as cytosine in the nucleic acids then 5-methyluracil or thymine possibly results by the cleavage of a corresponding base, namely, 5-methylcytosine (formula I.) or the isomeric 2-amino-5-methyl-6-oxypyrimidine (III.):

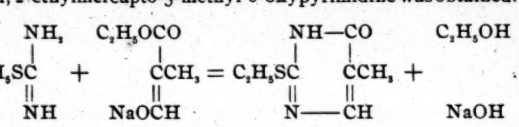


I. II. III.

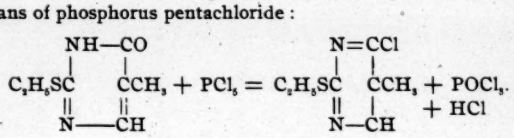
We therefore decided to prepare these new bases and to compare their properties with those of cytosine. In this paper we describe 5-methylcytosine and in a later paper by Dr. Johnson and Mr. S. H. Clapp, the compound represented by formula III. will be discussed. We prepared the compound represented by formula I. as follows: The ethyl bromide addition-product of thiourea was treated with one molecular

[1] This JOURNAL, **29**, 494 (1903).

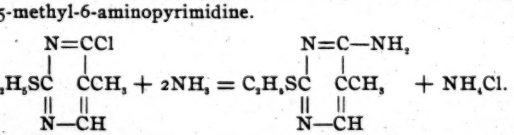
proportion of alkali in aqueous solution and the sodium salt of ethyl formylpropionate added. On standing, then warming for a few minutes, cooling, and acidifying with acetic acid, 2-ethylmercapto-5-methyl-6-oxypyrimidine was obtained.



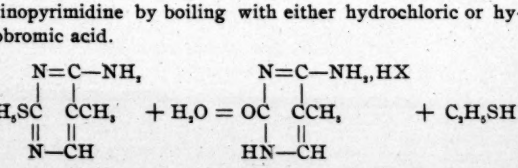
The 2-ethylmercapto-5-methyl-6-oxypyrimidine was converted into 2-ethylmercapto-5-methyl-6-chlorpyrimidine by means of phosphorus pentachloride:



When 2-ethylmercapto-5-methyl-6-chlorpyrimidine was heated with strong alcoholic ammonia it gave 2-ethylmercapto-5-methyl-6-aminopyrimidine.



Finally the 2-ethylmercapto-5-methyl-6-aminopyrimidine was converted into the halogen hydride salt of 2-oxy-5-methyl-6-aminopyrimidine by boiling with either hydrochloric or hydrobromic acid.



5-Methylcytosine separates from water with a half molecule of water of crystallization while cytosine crystallizes with 1 molecule of water.

5-Methylcytosine is about five times as soluble in water as cytosine and ten or eleven times as soluble as thymine or uracil and it is readily decomposed into thymine by 20 per cent sulphuric acid at 150°. It is probable that in the decomposition of the nucleic acids by the prolonged action of hot acids this base would, if present, be converted entirely into thymine.

One of the most characteristic properties of 5-methylcytosine is its tendency to form the so-called abnormal or basic 2 : 1 salts with hydrochloric and hydrobromic acids. When ammonia in excess is added to a solution of cytosine in these acids, free cytosine usually separates. In the case of 5-methylcytosine in the halogen hydride acids the precipitate generally consists of the salt $(C_5H_7ON_3)_2HX.H_2O$.

Cytosine gives a 1 : 2 salt with concentrated hydrochloric acid; 5-methylcytosine gives a 1 : 1 salt under the same conditions.

The picrate of 5-methylcytosine, which forms yellow, long, slender, needle-like prisms, has the same solubility in water as cytosine picrate.

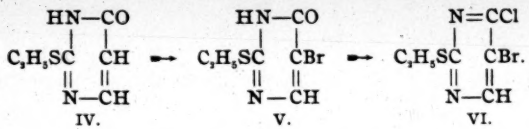
5-Methylcytosine is precipitated by phosphotungstic acid; it therefore differs from the new base prepared by Kutscher[1] from thymus nucleic acid.

An attempt was made to prepare 2-oxy-5,6-diaminopyrimidine by brominating 2-ethylmercapto-6-oxypyrimidine[2] (IV.), chlorinating in the 6-position by means of phosphorus pentachloride, and then attempting to replace both of the halogens by the amino group in this compound (VI.) by means of alcoholic ammonia at a high temperature. It was found that the chlorine could be replaced by the amino group with ease, but that the bromine was far more firmly bound, the compound VII. being almost quantitatively obtained. This was converted into 5-bromcytosine (VIII.) and this compound then heated with aqueous ammonia, but here again the desired diamino derivative was not obtained. These results recall the similar behavior of 4-methyl-6-amino-5-brompyrimidine de-

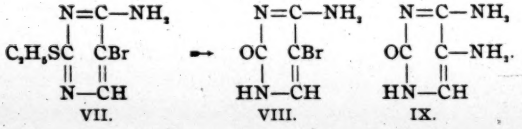
[1] Ztschr. physiol. Chem., **38**, 173 (1903).

[2] Wheeler and Merriam: This JOURNAL, **29**, 484 (1903).

scribed by Gabriel and Colman.[1] This substance did not react with ammonia without complete decomposition.

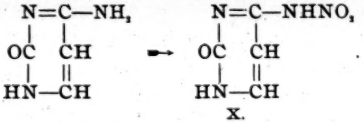


IV. V. VI.



VII. VIII. IX.

The action of nitric acid was next tried on cytosine, whereupon it was found that nitration took place; the compound formed was not a 5-nitro derivative, but rather the nitramide whose structure is, in all probability, shown by formula X.



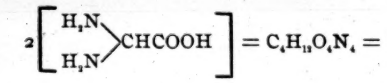
X.

The formation of similar nitramide derivatives in the pyrimidine series has been observed by Gabriel and Colman.[2]

Work on the preparation of oxydiaminopyrimidines will be continued here. These particular pyrimidine compounds are of special interest not only because Kutscher[3] believes that he has isolated such a compound, in the form of its picrate, from yeast, but also on account of the fact that if an oxydiaminopyrimidine is found to crystallize or combine with 3 molecules of water it would then have the same percentage composition as Drechsel's so-called diaminoacetic acid[4] and perhaps the substances would prove to be identical. Willstätter[5] has shown that diaminoacetic acid, if capable of ex-

[1] Ber. d. chem. Ges., 34, 1239 (1901).
[2] *Ibid.*, 34, 1240-1241 (1901).
[3] Ztschr. physiol. Chem., 38, 176 (1903).
[4] Beilstein: Handbuch I., 1194.
[5] Ber. d. chem. Ges., 35, 1378 (1902).

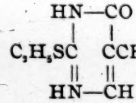
istence, would not agree in properties with Drechsel's compound. In other words, Drechsel's compound might be a pyrimidine derivative as follows:



$$2\left[\begin{matrix}H_2N\\ \\ H_2N\end{matrix}\!\!>\!CHCOOH\right] = C_4H_{12}O_4N_4 = C_4H_6ON_4 + 3H_2O.$$

EXPERIMENTAL PART.

2-Ethylmercapto-5-methyl-6-oxypyrimidine,



$C_2H_5SC\;\;CCH_3$.—When a mixture of 150 grams of ethyl propionate and 135 grams of ethyl formate, in somewhat over 2 volumes of dry ether, were slowly dropped upon 34 grams of sodium, about 125 grams of crude sodium ethylformylpropionate[1] were obtained. In using this salt and also sodium ethylformyl acetate to condense with the alkyl halide addition-products of thiourea we[2] employed the substances in molecular proportions. It has since been found in both cases that only about one-half, or less, of the quantity of the salt taken reacts in the desired manner, and therefore that one-half the calculated quantity of pseudoethylthiourea hydrobromide gives about the same, if not a better, yield of pyrimidine as when the substances are used in molecular proportions. From 125 grams of the salt and 150 grams of the ethyl bromide addition-product of thiourea we obtained, in the manner already described, 39.5 grams of this mercaptopyrimidine. This is 28 per cent of the calculated. In another experiment 90 grams of the sodium salt with 87 grams of the thiourea addition-product gave 20.5 grams of the pyrimidine derivative, or 26 per cent of the theoretical amount.

2-Ethylmercapto-5-methyl-6-oxypyrimidine crystallizes from hot water in the form of long, slender prisms. It is very sol-

[1] Wislicenus: Ber. d. chem. Ges., **20**, 2933 (1887).

[2] This JOURNAL, **29**, 487; *Ibid.*, **29**, 496 (1903).

uble in alcohol and it melts at 158° to 159°. A nitrogen determination gave :

	Calculated for $C_7H_{10}ON_2S$.	Found.
N	16.47	16.78

2-Ethylmercapto-5-methyl-6-chlorpyrimidine, $C_2H_5SC(N{=}CCl){-}CCH_3{=}N{-}CH$. — Phosphorus pentachloride reacts less smoothly with the above mercapto derivative than it does with 2-ethylmercapto-6-oxypyrimidine. When the mixture of the substances in molecular proportions is warmed on the steam-bath, hydrogen chloride is evolved, and, on cooling, the material solidifies to a deliquescent, crystalline cake. This is apparently a double compound of the chlorpyrimidine and phosphorus oxychloride. If an attempt is made to distil this directly in a vacuum, decomposition takes place and the material turns black. If, however, the cake is transferred to crushed ice it liquefies, and on shaking out with ether and drying, the resulting oil can then be distilled under diminished pressure. In one experiment, 35 grams of the mercapto-oxy derivative and 43.5 grams of phosphorus pentachloride gave 24 grams of the distilled chloride, which is a yield of 61.8 per cent of the calculated. In another, 20 grams gave 14.5 grams of the chloride or 65 per cent of the calculated.

2-Ethylmercapto-5-methyl-6-chlorpyrimidine boils at 146° to 147° at 17 mm. pressure, and at 157° to 159° at 25 to 26 mm. pressure. It was thus obtained as a colorless, almost odorless, oil, which did not solidify in a freezing-mixture.

2-Ethylmercapto-6-chlorpyrimidine, prepared and purified in a similar manner, was found to boil at 135° at 24 mm. pressure.

A nitrogen determination in the case of 2-ethylmercapto-5-methyl-6-chlorpyrimidine, boiling at 157° to 159°, gave :

	Calculated for $C_7H_9N_2SCl$.	Found.
N	14.85	15.00

2-Ethylmercapto-5-methyl-6-aminopyrimidine,

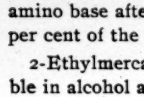
.—In the preparation of this amino derivative, and also in the case of mercaptocytosine, the alcoholic ammonia used should be thoroughly saturated (in the cold), as otherwise an ethoxy derivative is formed instead of an amino compound.

We found that 13 grams of the above chloride and about 75 cc. of alcoholic ammonia, when heated for three hours at 127°, gave 10 grams of amino derivative, or 86.9 per cent of the calculated, and also that 10.1 grams of the chloride, when heated for two hours at 124° to 130°, gave 6.6 grams of the amino base after crystallizing from 50 per cent alcohol, or 74 per cent of the theoretical amount.

2-Ethylmercapto-5-methyl-6-aminopyrimidine is very soluble in alcohol and quite difficultly soluble in water. It crystallizes from 50 per cent alcohol in the form of colorless, small, stout, six-sided tables and prisms, which melt at 96° to 97°. It has a tendency to separate from its solutions as an oil, which finally crystallizes. A nitrogen determination gave :

	Calculated for $C_7H_{11}N_3S$.	Found.
N	24.86	25.20

2-Ethylmercapto-6-ethoxypyrimidine,



.—2-Ethylmercapto-6-chlorpyrimidine was dissolved in alcohol and ammonia gas was passed in, the whole being heated on the steam-bath. After two hours, 7.5 grams of ammonium chloride had separated, the calculated quantity being 7.6 grams. On evaporating the alcoholic solution an oil was obtained which distilled at 137° to 138° at 18 mm.

pressure. A nitrogen determination agreed with the calculated for an ethoxy derivative :

	Calculated for $C_8H_{12}ON_2S$.	Found.
N	15.21	15.38

That this ethoxy derivative is not an intermediate product in the preparation of the amino compound was shown when on heating the oil with alcoholic ammonia for four or five hours at 150° to 175° it still remained unaltered.

This oil dissolves in dilute hydrochloric acid and is precipitated unaltered by ammonia. On boiling for several hours with strong acid it is converted quantitatively into uracil, melting at 338°. A nitrogen determination gave 25.4 per cent nitrogen, while the calculated is 25.0.

Cytosine Dihydrochloride.—When 2-ethylmercapto-6-aminopyrimidine is warmed on the water-bath for three or four hours with concentrated hydrochloric acid, then cooled, beautiful, stout, brittle, colorless, flat prisms separate. The following analysis shows that this material is an acid salt :

	Calculated for $C_4H_5ON_3.2HCl$.	Found.
Cl	38.58	39.09

This salt is analogous to the acid sulphate, $C_4H_5ON_3.H_2SO_4$, described by Kossel and Steudel.[1]

5-Methylcytosine Monohydrochloride (*Anhydrous*).—When 2-ethylmercapto-5-methyl-6-aminopyrimidine was warmed with hydrochloric acid, in a similar manner to the above, mercaptan was evolved and the crystals obtained were colorless tables and flat prisms, which, when dried over potassium hydroxide, showed signs of melting at about 270°, and melted with effervescence at 288°. The material was readily soluble in water. A chlorine determination showed that this was an anhydrous 1 : 1 salt.

	Calculated for $C_5H_7ON_3.HCl$.	Found.
Cl	21.98	21.99

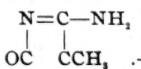
When 6.6 grams of the crude mercapto base were warmed

[1] Ztschr. physiol. Chem., **38**, 53 (1903).

on the steam-bath with hydrochloric acid until mercaptan ceased being evolved, the solution evaporated to dryness, and the residue dissolved in a little water, 0.7 gram thymine was obtained, the formation of this being due to the presence of an ethoxy derivative. When this 1 : 1 hydrochloride was dissolved in water and to the warm solution an excess of ammonia was added, microscopic colorless needles slowly separated. These were not very soluble in hot water and difficultly in cold. The material was recrystallized from water. The curious result was then observed that the material still contained chlorine and that we had to deal with a basic salt or, more probably, a mixture of free base and a hydrous basic salt. The analytical results agreed fairly with the calculated for $(C_5H_7ON_3)_2HCl.3H_2O$.

	Calculated.	Found.
Cl	9.4	9.09
H_2O	7.1	7.53

The water determination was made by heating the material to constant weight at 150°. When the 1 : 1 hydrochloride is boiled with water a basic salt also is obtained.

5-Methylcytosine,  .—In order to obtain the free base the above material was dissolved in water, the chlorine was removed by means of silver sulphate, the excess of silver was precipitated with hydrogen sulphide and the sulphuric acid by barium hydrate, the excess of the latter by carbon dioxide. The clear, colorless solution was then concentrated to a small volume, whereupon, on cooling, beautiful, flat, colorless, prismatic, brittle crystals separated that formed a sandy powder. When heated, the crystals gave off water above 100° and showed signs of melting at 240°, melting with effervescence at 270°; this is 50° lower than the melting-point of cytosine.

100 parts of water dissolved 4.5 parts of the base at 25°.

2.387 gram substance lost 0.1544 gram on heating to constant weight at 135° to 145° (I.).

	Calculated for $(C_5H_7ON_3)_2.H_2O$.	Found. I.	II.
H_2O	6.71	6.46	6.30

	Calculated for $C_5H_7ON_3$.	Found.
N	33.60	33.44

Silver nitrate, added to an aqueous solution of this base, gives a gelatinous white precipitate soluble in an excess of ammonia and in nitric acid. The ammonia solution can be boiled without reduction.

Mercuric chloride gives a white precipitate in dilute solutions of the base; this dissolves on heating and separates again, on cooling, possibly in crystalline form.

Phosphotungstic acid gives a white precipitate insoluble in dilute hydrochloric or sulphuric acids.

5-Methylcytosine Monohydrochloride (*Hydrous*).—An attempt was made to prepare a dihydrochloride of the methyl base by dissolving the above 1 : 1 salt in water and then saturating the solution with hydrogen chloride. The solution was then allowed to evaporate spontaneously in a desiccator over sulphuric acid, whereupon small, stout, transparent prisms or rhombohedrons separated. The analysis agreed with the calculated for the formula $C_5H_7ON_3.HCl.2H_2O$.

	Calculated.	Found.
H_2O	18.2	18.0
Cl	21.98	22.0

On keeping these crystals in a closed tube they became opaque.

5-Methylcytosine Basic Hydrochloride.—Experiments were made to determine whether or not 5-methylcytosine gives a 2 : 1 and a 3 : 1 salt by mixing solutions of the base and the above hydrochloride in the required proportions. It was found that when a strong solution of the base (0.12 gram) was mixed with a warm solution of the hydrochloride (0.153 gram) in a little water a precipitate was formed immediately. This dissolved on boiling, and on cooling, little balls of mi-

nute needles or prisms separated. When dried over calcium chloride the salt was found to have the composition represented by Analysis I. When two molecular proportions of the base to one of the hydrous hydrochloride were used the same basic salt was obtained. For example: 0.15 gram of the hydrochloride was dissolved in 2.5 cc. of water and 2.2 grams of the base were dissolved in 5 cc. of water. On mixing these solutions at ordinary temperature a gelatinous mass separated, so bulky that the test-tube could be inverted without loss of the contents. On warming, the material dissolved and on cooling small prisms separated. Analysis II.:

	Calculated for $(C_5H_7ON_3)_2HCl.H_2O$.	Found. I.	II.
H_2O	5.9	6.2	5.9
Cl	11.6	11.3	

This hydrous basic salt is analogous to the hydrous basic cytosine sulphate frequently obtained by Levene.[1] This salt has the formula $(C_4H_5ON_3)_4H_2SO_4.2H_2O$.

The corresponding anhydrous basic sulphate has been described by Kossel and Steudel.[2]

A neutral sulphate of cytosine has also been described by Levene.[3]

5-Methylcytosine Basic Hydrobromide.—Ten grams of 2-ethylmercapto-5-methyl-6-aminopyrimidine were boiled with 15 cc. of strong hydrobromic acid for four hours. The acid was then evaporated, whereupon beautiful, large, stout prisms separated. These crystals were very soluble in water. Upon adding strong ammonia to the warm aqueous solution an immediate precipitate in the form of a fine, crystalline powder was obtained. The yield was 7 grams. This precipitate was difficultly soluble in water. When heated it sintered at about 295° and then decomposed with effervescence at 319°–320°. This material was boiled for some time with strong aqueous ammonia; it nevertheless still contained bromine, and on analysis the following results were obtained: The air-dried salt lost water very slowly below 150°, dried at 175°–185°.

[1] Ztschr. physiol. Chem., **39**, 7; **39**, 135, 481 (1903).
[2] *Ibid.*, **38**, 53 (1903).
[3] *Ibid.*, **37**, 405 (1903).

0.4838 gram lost 0.0290 gram water or 5.9 per cent. The calculated for $(C_5H_7ON_3)_2HBr.H_2O$ is 5.1 per cent. The dried residue then gave the following results:

	Calculated for $(C_5H_7ON_3)_2HBr$.	Found.
C	36.25	36.20
H	4.53	4.85
N	25.37	25.68

This basic salt was then treated with silver sulphate as described above in the case of the basic hydrochloride. The free base obtained agreed in every particular with the preparation from the basic hydrochloride. A water determination is given above (II.).

When 0.7 gram of this basic salt was heated at 150°–160° for three hours with 20 per cent sulphuric acid, 0.20 gram of thymine, melting at 325°–326°, was obtained. Forty per cent of the material was therefore hydrolyzed.

Acetyl-5-methylcytosine, prepared by dissolving the base in acetic anhydride, forms needles from water. The material did not melt or effervesce below 290° although it showed signs of decomposition at about 255°.

5-Methylcytosine Picrate.—When 0.238 gram of the base in a little water was mixed with 40 cc. of a saturated aqueous solution of picric acid, an immediate precipitate was formed. This dissolved on boiling and adding more water; on cooling, long, slender, bright yellow, needle-like prisms separated. On heating, this material showed signs of change at about 250°–255° and from then on got darker until at 286° it decomposed with effervescence. A certain mixture of this material with pure cytosine picrate began to change color, on heating, at 255° and melted with effervescence at 273°. It is noteworthy that this picrate has the same solubility in water as cytosine picrate.[1] One hundred parts of water dissolve 0.07 part of the salt.

The *chloroplatinate* is quite soluble in water. The aqueous solution, on standing in a desiccator, deposits small orange-colored rosettes.

[1] Wheeler and Johnson: *Loc. cit.*

2-Ethylmercapto-5-brom-6-oxypyrimidine,

$C_2H_5SC\ \ CBr$. — When 2-ethylmercapto-6-oxypyrimidine (5.55 grams) was dissolved in three parts of glacial acetic acid and one molecular proportion of bromine was added (6 grams), a mass of needles separated; these were washed with water and dried; the weight was 7.45 grams or 89 per cent of the calculated. In another experiment 16 grams of the mercapto compound gave 22.1 grams of the brom-derivative or 91.7 per cent of the theoretical amount. This material was difficultly soluble in water, but more readily soluble in alcohol, from which solution beautiful, colorless, needle-like prisms were obtained. These, on heating, showed signs of change at about 184° and melted completely without effervescence at 189°. A nitrogen determination gave:

	Calculated for $C_4H_7ON_2SBr$.	Found.
N	11.9	12.1

An excess of bromine does not effect further substitution in the cold. When the substance is boiled with hydrochloric acid, 5-bromuracil is obtained.[1] Dr. Merriam found that when this latter substance was heated with aqueous ammonia to 180°, 5-aminouracil resulted, the compound being identified by means of its picrate.[2]

The action of alcoholic ammonia on the brommercapto compound at temperatures between 140° and 200° did not lead to a smooth result.

2-Ethylmercapto-5-brom-6-chlorpyrimidine,

$C_2H_5SC\ \ CBr$.—2-Ethylmercapto-5-brom-6-oxypyrimidine (21 grams) and phosphorus pentachloride (18.6 grams) were mixed and warmed on the steam-bath. After standing over

[1] Wheeler and Merriam: *Loc. cit.*

[2] Behrend and Grünwald: Ann. Chem. (Liebig), 309, 258 (1899).

night the phosphorus oxychloride was removed by distilling under diminished pressure and the remaining oil poured off from a small amount of resin; this was washed with a little ether. The weight of crude oil then obtained, on evaporating the ether, was 20.5 grams or 92 per cent of the calculated. On standing, this oil solidified in the form of soft, talc-like, colorless plates which, when pressed on paper, melted at about 27°. A nitrogen determination then gave:

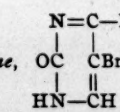
	Calculated for $C_6H_4N_2SClBr$.	Found.
N	11.04	11.06

2-Ethylmercapto-5-brom-6-aminopyrimidine,

.—The above chlorobromide (19 grams) was heated with alcoholic ammonia (40 cc. cold saturated solution) to 160° for four hours. On cooling, a crystalline mass separated with little or no color. Water was added to the contents of the tube; the material that separated, on drying, weighed 15.7 grams or 89.7 per cent of the calculated. The substance was readily soluble in hot alcohol and difficultly in cold, and very difficultly soluble in hot water. On crystallizing from alcohol it formed beautiful, colorless prisms which melted sharply at 123° to 124°. The material contained sulphur and bromine, and a nitrogen determination gave the following result:

	Calculated for $C_6H_5N_3SBr$.	Found.
N	17.9	17.8

5-Bromcytosine,

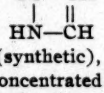


.—Twelve grams of the above mercapto compound were boiled with 50 cc. of concentrated hydrochloric acid for four or five hours. The acid was then evaporated and the residue crystallized from water,

whereupon minute prisms of the hydrochloric acid salt were obtained. To the hot solution of this material strong ammonia was added in excess, and then, on cooling, bunches of needle-like prisms of the free base separated. The yield was 7.5 grams, or 77.3 per cent of the calculated, more being obtained from the mother-liquor. When the pure base, crystallized from water, was heated it gave no definite melting-point but decomposed rapidly above 235°. A nitrogen determination gave:

	Calculated for $C_5H_4ON_3Br$.	Found.
N	22.10	22.11

When this material was heated at 155° to 190° with concentrated aqueous ammonia for four hours, the products were a black resin and a brick-red amorphous powder, the material having undergone decomposition.

Nitrocytosine,  .—Two grams of anhydrous cytosine (synthetic), 4. cc. of concentrated nitric acid, and 4 cc. of concentrated sulphuric acid were mixed and warmed for a few minutes at 85°. The mixture was then poured upon crushed ice and neutralized (slight excess of ammonia added). The crystalline material that separated weighed 1.8 grams, or 64 per cent of the calculated. In another experiment 2 grams of cytosine and 6 cc. of each acid gave 1.7 grams of the crude nitro compound. This nitro compound is very difficultly soluble in water, from which it separates in the form of colorless, minute needles. These are readily soluble in strong ammonia. When heated, the material begins to turn brown at about 280°, decomposes rapidly above 300°, but does not melt or effervesce at 350°. A nitrogen determination gave the following result:

	Calculated for $C_4H_4O_3N_4$.	Found.
N	35.89	35.80

When an attempt was made to reduce this material with

tin and hydrochloric acid the only crystalline material obtained was ammonium chloride. According to Behrend and Grünwald[1] 5-nitrouracil gives 80 per cent of the calculated of aminouracil, when reduced in ammoniacal solution by means of aluminium amalgam. An attempt to reduce our nitrocytosine in a similar manner gave nothing but amorphous yellow and blue-black decomposition-products. For this reason the above nitramide structure is assigned to the compound.

NEW HAVEN, CONN., Feb. 1, 1904.

ON THE REDUCTION OF NITRATES BY CERTAIN PLANT EXTRACTS AND METALS, AND THE ACCELERATING EFFECT OF CERTAIN SUBSTANCES ON THE PROGRESS OF THE REDUCTION.

BY J. H. KASTLE AND ELIAS ELVOVE.[2]

The fact that both higher and lower plants are able to construct proteids out of nitrates naturally suggests that the nitrates must suffer a gradual reduction in this phase of nitrogen metabolism. It has been observed that certain plants, such as the tobacco, contain so much nitrate in their leaves that this crystallizes out on drying, and the writers of this communication have obtained excellent tests for nitrite on several specimens of air-dried tobacco. Whether this nitrite is of bacterial origin or results from reducing changes naturally occurring in the leaf itself we have not yet been able to determine. Indeed, chemists seem to be divided in opinion as to whether nitrites ever occur in the living plant. According to Molisch,[3] for example, nitrites could not be detected in one hundred different plants which he examined. According to this observer all statements as to the presence of nitrites in plants are based on experimental errors and incorrect interpretation of results. According to him plants possess the power of reducing nitrites with surprising rapidity, whereas nitrates will remain for weeks, and even months, in vegetable cells before

[1] *Loc. cit.*

[2] Research Assistant in the Carnegie Institution of Washington.

[3] Monatsh. Chem., 8, 237–259.

they are destroyed. On the other hand, Modderman[1] was able to detect nitrites in the exudations of the fuchsia, by means of starch, potassium iodide, and sulphuric acid, and also by means of other reagents. Recently also K. Aso[2] has called attention to the occurrence of nitrites in the buds of the potato and *Sagittaria sagittæfolia*. In view of these facts it seemed of interest to determine whether the aqueous extracts of certain plants had the power of reducing nitrates to nitrites. In other words, this investigation had for its object to determine whether any of the higher plants can bring about those changes ordinarily included under the head of denitrification.

According to Berthelot,[3] in the leaves and all such organs of the plant, the conditions which tend to the production of nitrates by oxidation of nitrogenous substances and those which tend toward the reduction of nitrates, result ultimately in a state of unstable equilibrium (*equilibre mobile*) with respect to the assimilation of nitrogen. The agents which produce such results being, on the one hand, those which bring about reduction, and on the other, those which effect oxidation in the living organism, that is to say, they thus resemble the soluble ferments, or more generally oxidizable principles, which by their oxidation can effect the oxidation of other substances. The existence of such a substance in the potato was long ago pointed out by Schönbein and confirmed by Kastle and Loevenhart.[4] That is to say, the potato contains easily oxidizable substances, which on exposure to the air are converted into oxidases by processes of autoxidation. It therefore occurred to us that the potato would probably prove to be a convenient plant material with which to begin the investigation.

Preparation and Denitrifying Properties of the Aqueous Extract of the Potato Tuber.

The aqueous extract of the potato was usually prepared by

[1] Ann. Agronom., 14, 423–424.
[2] Bull. Agric. Coll., Tokyo Imp. Univ., 5, 487.
[3] Berthelot: Chimie Vegetale et Agricole, 3, 183, 469, 507.
[4] This JOURNAL, 26, 539–566.

cutting up the inner portion of a potato tuber into small pieces and macerating rapidly in a mortar with 100 to 150 cc. of water. Generally the extract thus obtained was not filtered, but simply decanted off from the larger pieces and immediately used in the experiment, for the reason that filtration seemed to diminish the activity of the extract, and also because this would result in a longer exposure to the air, and this likewise seems to diminish its denitrifying powers. An extract which was allowed to stand for two days under antiseptic conditions was found at the end of that time to have completely lost its activity. In most cases 10 cc. of the fresh extract were employed. To this was added 0.5 cc. of toluene as antiseptic, and finally, 2 cc. of a solution of pure sodium, potassium, or ammonium nitrate of known concentration. The tube containing the mixture was then tightly corked and shaken, and allowed to stand at a certain temperature, usually 40° C., for the desired interval, at the end of which time 2 cc. were pipetted out and tested for nitrite with Griess' sulphanilic acid reagent, and the amount of nitrite, if found present, determined colorimetrically. Control experiments were tried in all cases. In these the same aqueous extract was employed as that used in any particular series of experiments, the only difference being that in the control experiments the extract was boiled before the addition of the nitrate. The effect of temperature, of various antiseptics, of varying the concentration of nitrate and potato extract, on the progress of the reaction, have all been investigated. The results of our studies are given in the following pages.

The fact that an aqueous extract of the potato tuber can reduce a nitrate to a nitrite is apparent from the results reached in the following experiments:

Experiment 1.—A fresh potato extract was prepared. Two tubes received 10 cc. each of the non-filtered extract. To one of these was immediately added 0.5 cc. of toluene, while the other was heated to boiling for three minutes. It was then cooled, diluted with water to the original volume, and 0.5 cc. of toluene added to it also. To each tube there was then added simultaneously 5 cc. of a solution of pure sodium

nitrate containing 0.5 gram of the salt. Both tubes were then tightly corked and shaken, and allowed to remain at 40° C. for two hours. At the end of this time 2 cc. were pipetted out from each tube and tested for nitrite. The following results were obtained:

Two cc. of the active liquid contained nitrite equal to 0.120 cc. of the standard,[1] or a total of 0.16407 milligram.

Two cc. of the boiled liquid contained no nitrite.

Experiment 2.—This was similar in every respect to the above, except that the 5 cc. of sodium nitrate solution added to each tube contained only 0.025 gram of the salt. After allowing both tubes to remain at 40° C. for two hours the following results were obtained:

Two cc. of the active liquid contained nitrite equal to 0.100 cc. of the standard, or a total of 0.13673 milligram.

Two cc. of the boiled liquid contained no nitrite.

Experiment 3.—This was similar in every respect to Experiment 1, except that the amount of antiseptic added to each tube was increased to 1 cc. After standing for two hours at 40° C. the following results were obtained:

Two cc. of the active liquid contained nitrite equal to 0.085 cc. of the standard, or a total of 0.11622 milligram.

Two cc. of the boiled liquid contained no nitrite.

Experiment 4.—This differed from Experiment 1 only in that the extract was quickly filtered once. After two hours' standing at 40° C. the following results were obtained:

Two cc. of the active liquid contained nitrite equal to 0.075 cc. of the standard, or a total of 0.10254 milligram.

Two cc. of the boiled liquid contained no nitrite.

It should be noted in this connection that our earlier work along this line led invariably to negative results. It was found out afterwards, however, that the failure was due partly to the fact that the nitrate solution employed was too dilute. It became of interest, therefore, to determine the effect of varying the amount of nitrate on the progress of the reduction. The experimental data and results obtained are given in Table I.

[1] One cc. of the standard employed contained 0.18231 milligram of sodium nitrite.

Table I.

The indicated amount of sodium nitrate was added to 20 cc. of fresh potato extract containing 0.5 cc. of toluene as antiseptic.

Time, three hours. Temperature, 30° C.

No. of experiment.	Amount of sodium nitrate in grams.	Nitrite in 2 cc. expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in milligrams.
1	0.001	0.080	0.800	0.14584
2	0.010	0.100	1.000	0.18231
3	0.050	0.145	1.450	0.26434
4	0.250	0.200	2.000	0.36462
5	1.250	0.290	2.900	0.52869
6	6.250	0.340	3.400	0.61985
7	10.000	0.200	2.000	0.36462

It will be seen from an examination of the results given in Table I. that the amount of nitrite formed in three hours at 30° C. by the action of 20 cc. of the aqueous extract of the potato on sodium nitrate increases, more or less regularly, with increasing amounts of nitrate until a maximum is reached with 6.25 grams of sodium nitrate, the amount of nitrite formed being more than when 10 grams of the salt are used. Hence the effect of increasing the amount of nitrate is to increase the amount of nitrite formed, so long as the amount used is below a certain maximum, after which the effect is to retard the reduction. This is probably to be explained by the poisonous action of the salt at greater concentration.

The Effect of Temperature on the Reduction of Sodium Nitrate by Aqueous Extracts of the Potato.

The effect of temperature on the progress of the reaction under investigation has also been studied. The experimental data and results are given in Table II. :

Table II.

Ten cc. of potato extract containing 0.5 cc. of toluene as antiseptic, 5 cc. of a sodium nitrate solution containing 0.5 gram of the salt.

Time, two and one-half hours.

No. of experiment.	Temperature.	Nitrite in 2 cc. expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in milligrams.
1	0°– 5° C.	0.002	0.015	0.00273
2	10°–15° C.	0.005	0.037	0.00674
3	15°–20° C.	0.010	0.075	0.01367
4	30° (room temp.)	0.100	0.750	0.13673
5	40°–45° C.	0.130	0.975	0.17775
6	45°–50° C.	0.100	0.750	0.13673
7	50°–55° C.	0.060	0.450	0.08203
8	55°–60° C.	0.055	0.412	0.07511
9	60°–70° C.	0.050	0.375	0.06836
10	75°–80° C.	0.001	0.007	0.00127
11	100° C.	0.000	0.000	0.00000

It will be observed that the amount of nitrite formed increases with rise of temperature, reaching a maximum at 40° to 45° C., above which it begins to decrease in amount, becoming nil at 100° C. The optimum temperature of the reaction is, therefore, between 40° and 45° C. We thus see that the change under investigation is similar to other biochemic and zymotic processes, so far as the influence of temperature is concerned.

The Effect of Increasing Amounts of the Potato Extract on the Reduction.

The effect of increasing the quantity of the potato extract on the reduction here under investigation has also been tried. The conditions and results of these experiments are given in Table III. :

Table III.

Number of cubic centimeters of potato extract varying as indicated in column 2 of table; 2 cc. of 50 per cent solution of sodium nitrate; 0.5 cc. of toluene as antiseptic; the whole made up to 28 cc. with distilled water.

Time, twelve hours. Temperature, 30° C.

No. of experiment.	Potato extract in cc.	Nitrite in 2 cc. expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in milligrams.
1	5	0.050	0.700	0.12761
2	10	0.090	1.260	0.22971
3	15	0.120	1.680	0.30628
4	20	0.140	1.960	0.35733
5	25	0.150	2.100	0.38286

It will be observed that the amount of nitrite formed increases with the amount of the potato extract present. With small amounts of the extract, as will be observed from Experiments 1 and 2, the amount of nitrite formed is nearly exactly proportional to the quantity of extract employed. Thus the amount of nitrite formed in Experiment 2 was 0.22971 milligram, whereas if the amount formed were exactly proportional to the amount of extract used, it should have been 0.25522 milligram. On the other hand, the amount of nitrite formed when 25 cc. of the potato extract was employed was only 0.38286 milligram, whereas, had it been exactly proportional to the quantity of the extract employed it should have been 0.63805 milligram. Hence the reduction by increasing quantities of the reducing substance in the potato tends toward a maximum, which is probably not further augmented by increasing amounts of the potato extract. The same is true of the action of certain, if not all, of the soluble ferments.

On the Inhibiting Action of Certain Substances on the Reduction of Nitrates by the Potato Extract.

It is known that most biochemical processes and zymotic changes are inhibited, and often completely checked, by the

presence of certain poisons. It therefore occurred to us to try the effect of certain poisonous substances on the change under investigation. The names of the substances tried, together with the quantities employed, and the other conditions of the experiments, as well as the results, are given in Table IV. :

Corresponding controls showed no nitrite. It was also ascertained that none of the substances mentioned in the above table interfere with the nitrite test when present in the proportions used in the above experiments. It is evident from these results that the reduction of nitrates by the aqueous extracts of the potato is completely inhibited or checked by mercuric chloride, chloroform, resorcinol, and also by alkalies. Its activity is also greatly lessened by phenol, phenylhydrazine hydrochloride, sodium hypophosphite, hydroquinone, hydroxylamine hydrochloride, benzenesulphinic acid, sodium fluoride, and hydrocyanic acid.

Accelerating Effect of Certain Substances on the Progress of the Reduction.

In order to exclude denitrifying organisms we were careful in our earlier work to use considerable amounts of toluene as antiseptic. On comparing the activity of a certain extract alone and in the presence of toluene, it was observed that the toluene apparently accelerated the reduction, that is, more nitrite was formed in the presence than in the absence of this substance, and yet toluene alone has no power to reduce a nitrate, under the conditions of our experiments. This led us to study the effect of a large number of substances on the progress of the reaction. The name of the substance tested, and the amount of it employed, together with the general conditions of the experiments and the results obtained, are given in Table V.

Corresponding controls showed no nitrite.

It will be observed that quite a considerable number of substances, notably alcohols and aldehydes, have the power of greatly accelerating the reduction of sodium nitrate by potato extract. The accelerating action of benzyl alcohol and benzaldehyde especially is very remarkable. By comparing

Table IV.

Ten cc. potato extract; 2 cc. of a 50 per cent. solution of sodium nitrate; 5 cc. water, or solution containing the indicated amount of the substance.

Time, one hour. Temperature, 40° C.

Substance.	Amount of substance used.	Nitrite in 2 cc. expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in milligrams.
None		0.030	0.255	0.04649
Hydrocyanic acid	0.1 cc. N/10 solution	0.025	0.212	0.03865
" "	0.5 cc. N/10 "	0.018	0.153	0.02789
Sodium fluoride	1 part NaF : 1000 parts liquid	0.015	0.127	0.02315
" "	1 part NaF : 500 parts liquid	0.010	0.085	0.01550
Hydrocyanic acid	1 cc. N/10 solution	0.010	0.085	0.01550
Benzenesulphinic acid	1 cc. 1 per cent solution	0.010	0.085	0.01550
Hydroxylamine hydrochloride	1 part : 1000 parts liquid	0.005	0.042	0.00766
Hydroquinone	0.1 gram	0.005	0.042	0.00766
Sodium hypophosphite	1 cc. 20 per cent solution	0.003	0.025	0.00456
Phenylhydrazine hydrochloride	5 cc. N/100 solution	0.002	0.017	0.00310
Phenol	0.1 cc.	0.002	0.017	0.00310
Resorcinol	0.1 gram	0.000	0.000	0.00000
Chloroform	0.5 cc.	0.000	0.000	0.00000
Mercuric chloride	1 part : 1000 parts liquid	0.000	0.000	0.00000
Lime-water	Sufficient to neutralize acidity	0.000	0.000	0.00000
Sodium hydroxide	1 cc. N/10 solution	0.000	0.000	0.00000

Table V.

Ten cc. potato extract; 2 cc. 50 per cent solution of sodium nitrate; 1 cc. water, or solution containing the indicated amount of accelerator.

Time, one hour. Temperature, 40° C.

Accelerator.	Amount of accelerator.	Nitrite in 2 cc. expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in millig'ms.
None		0.025	0.1625	0.02962
Methyl alcohol	0.05 cc.	0.030	0.1950	0.03555
Acetone	0.1 "	0.040	0.2600	0.04740
Bromal	0.1 gram	0.045	0.2925	0.05332
Chloral	0.1 "	0.050	0.3250	0.05925
Eugenol	0.1 cc.	0.050	0.3250	0.05925
Propyl alcohol	0.1 "	0.065	0.4225	0.07702
Formaldehyde	1 cc. of 4 per cent solution	0.075	0.4875	0.08887
Salicylic aldehyde	0.1 cc.	0.075	0.4875	0.08887
Glucose	0.1 gram	0.075	0.4875	0.08887
Allyl alcohol	0.1 cc.	0.080	0.5200	0.09480
Toluene	0.5 "	0.085	0.5525	0.10072
Ethyl alcohol	1 cc. 90 per cent	0.090	0.5850	0.10665
Thymol	0.5 cc. 10 per cent alcoholic solution	0.090	0.5850	0.10665
o-Nitrobenzaldehyde	0.1 gram	0.090	0.5850	0.10665
Levulose	0.1 cc.	0.090	0.5850	0.10665
Vanillin	0.1 gram	0.120	0.7800	0.14220
Acetaldehyde	1 cc. 4 per cent solution	0.180	1.1700	0.21330
Benzaldehyde	0.1 cc.	0.600	3.9000	0.71100
Benzyl alcohol	0.1 "	0.650	4.2250	0.77025

the quantities of nitrite produced when 0.1 cc. of these substances were present with that formed by the potato extract alone, it will be seen that each of these substances has increased the amount of nitrite formed from twenty-five to thirty times. The fact, too, that many of these accelerators are poisonous and antiseptic in their general properties enhances the interest attaching to their action on the change under consideration, and the further fact that the control experiments differing only from the other experiments in that the extract of the potato had been boiled previous to use, gave no test for nitrite shows that these various accelerators alone have not the power to reduce nitrates, under the conditions here employed. The further significance of these results will be discussed in a subsequent part of this communication.

On the Effect of Temperature on the Reduction of Nitrates by the Potato Extract, in the Presence of an Accelerator.

The effect of the accelerator on the reduction of nitrates by the potato extract has been studied under a variety of conditions. Among other things, the effect of temperature on the reduction in the presence of an accelerator has been investigated, using formaldehyde, acetic aldehyde, benzaldehyde, ethyl and benzyl alcohols as accelerators. The general conditions of the experiments, together with the results obtained, are given in Table VI. :

A comparison of the results in Table VI. with those given in Table II. goes to show that the effect of temperature on the reduction is essentially the same, both in the presence and absence of an accelerator. The optimum temperature of the reduction in the absence of an accelerator was found to be between 40° and 45° C., and in the presence of benzyl and ethyl alcohol and acetic and benzoic aldehydes the optimum temperature of the reaction was also found to be 40° C., whereas in the presence of formic aldehyde it was found that the largest amount of nitrite was formed at 60° C. The fact that very small amounts of nitrite were produced even at 100° C., in the presence of certain of these accelerators, is doubtless to be explained on the ground that at these higher tem-

Table VI.

10 cc. potato extract; 2 cc. 35 per cent solution of potassium nitrate; 1 cc. water, or solution containing the indicated amount of accelerator.

Time, one hour.

Temperature, C.	Accelerator.	Amount of accelerator.	Nitrite in 2 cc expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in milligrams.
0	Formaldehyde	1 cc. N/5 solution	0.040	0.260	0.05826
20	"	1 " N/5 "	0.085	0.552	0.12370
40	"	1 " N/5 "	0.120	0.780	0.17480
60	"	1 " N/5 "	0.150	0.975	0.21850
80	"	1 " N/5 "	0.010	0.065	0.01457
100	"	1 " N/5 "	0.005	0.032	0.00727
0	Acetaldehyde	1 cc. 1 per cent solution	0.080	0.520	0.11653
20	"	1 " 1 " "	0.220	1.430	0.32046
40	"	1 " 1 " "	0.240	1.560	0.34960
60	"	1 " 1 " "	0.125	0.812	0.18197
80	"	1 " 1 " "	0.025	0.162	0.03630
100	"	1 " 1 " "	0.015	0.097	0.02174
0	Benzaldehyde	0.1 cc.	0.060	0.390	0.08740
20	"	0.1 "	0.220	1.430	0.32046
40	"	0.1 "	0.650	4.225	0.94682
60	"	0.1 "	0.090	0.585	0.13110
80	"	0.1 "	0.000	0.000	0.00000
100	"	0.1 "	0.000	0.000	0.00000

Table VI. (Continued).

Temperature C.	Accelerator.	Amount of accelerator.	Nitrite in 2 cc. expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in milligrams.
0	Ethyl alcohol	1 cc. 90 per cent solution	0.060	0.390	0.08796
20	"	1 " 90 " "	0.100	0.650	0.14494
40	"	1 " 90 " "	0.110	0.715	0.16015
60	"	1 " 90 " "	0.050	0.325	0.07276
80	"	1 " 90 " "	0.015	0.097	0.02174
100	"	1 " 90 " "	0.005	0.032	0.00717
0	Benzyl alcohol	0.1 cc.	0.095	0.617	0.13827
20	"	0.1 "	0.225	1.462	0.32763
40	"	0.1 "	0.675	4.387	0.98312
60	"	0.1 "	0.210	1.365	0.30590
80	"	0.1 "	0.085	0.552	0.12370
100	"	0.1 "	0.020	0.130	0.02914

NOTE.—Control experiments with each of the above accelerators, in which the boiled extract was employed instead of the active, were kept at the optimum temperature, without showing more than mere traces of nitrite.

peratures the accelerators themselves are rendered sufficiently active to reduce a very minute quantity of the nitrate.

Effect of Time on the Reduction of the Nitrate by the Potato Extract in the Presence of an Accelerator.

The effect of time on the reduction of the nitrate by the potato extract in the presence of certain accelerators has also been studied. The results obtained, together with all necessary data, are given in Table VII.

Control experiments with each of the accelerators employed above, gave none or only slight traces of nitrite.

It will be observed that the longer the substances are allowed to remain together the more nitrite is produced. However, the quantity of nitrite formed is not proportional to the time interval of the reaction. Proportionately a much larger quantity of nitrite is formed during the first hour than during either the second or the third. It is interesting to note, however, that in most cases the quantity of nitrite formed during the first two hours is almost an exact mean between that formed in one and three hours. This affords a convenient check on the results.

On the Effect of Increasing Amounts of Nitrate on the Reduction by Potato Extract in the Presence of Certain Accelerators.

The effect of increasing amounts of nitrate on the reduction of nitrate by the potato extract in the presence of formic aldehyde or benzyl alcohol as the accelerator has also been studied. The results of our observations upon this point, together with all necessary data, are given in Table VIII.

It will be observed that the amount of nitrite formed increases with increase in the quantity of nitrate present. This increase, however, is by no means proportional to the amount of nitrate present. If it were, we should have 640 times as much nitrite formed in the experiments with 32 grams of nitrate as were produced in those in which 0.05 gram was employed, whereas in reality only about 10 times as much was formed. Relatively more nitrite is formed, therefore, when small amounts of nitrate are present. This has also been observed to be the case where the reduction is affected by the potato

Table VII.

Ten cc. potato extract; 2 cc. 35 per cent solution of potassium nitrate; 1 cc. water, or solution containing the indicated amount of accelerator.

Temperature, 40° C.

Time in hours.	Accelerator.	Amount of accelerator.	Nitrite in 2 cc. expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in milligrams.
1	Formaldehyde	1 cc. N/5 solution	0.120	0.780	0.17480
2	"	1 " N/5 "	0.170	1.105	0.24763
3	"	1 " N/5 "	0.210	1.365	0.30590
1	Acetaldehyde	1 " 4 per cent solution	0.240	1.560	0.34960
2	"	1 " 4 " " "	0.375	2.437	0.54613
3	"	1 " 4 " " "	0.400	2.600	0.58266
1	Benzaldehyde	0.1 cc.	0.650	4.225	0.94682
2	"	0.1 "	0.800	5.200	1.16532
3	"	0.1 "	0.950	6.175	1.38382
1	Ethyl alcohol	1 cc. 90 per cent	0.110	0.715	0.16023
2	" "	1 " 90 " "	0.165	1.072	0.24024
3	" "	1 " 90 " "	0.175	1.137	0.25480
1	Benzyl "	0.1 cc.	0.675	4.387	0.98312
2	" "	0.1 "	0.845	5.492	1.23075
3	" "	0.1 "	0.975	6.337	1.42012

Table VIII.

Twenty cc. potato extract; amount of sodium nitrate used in each experiment given in column 3. In each experiment the volume was made up to 50 cc. with distilled water.

Time, one hour. Temperature, 40° C.

Accelerator.	Amount of accelerator.	Amount of nitrate in grams.	Nitrite in 2 cc. expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in milligrams.
Formaldehyde	1 cc. N/5 solution.	0.05	0.020	0.500	0.09115
"	1 " N/5 "	0.50	0.075	1.875	0.34183
"	1 " N/5 "	1.00	0.090	2.250	0.41019
"	1 " N/5 "	2.00	0.120	3.000	0.54693
"	1 " N/5 "	4.00	0.150	3.750	0.68366
"	1 " N/5 "	8.00	0.165	4.125	0.75202
"	1 " N/5 "	16.00	0.190	4.750	0.86597
"	1 " N/5 "	32.00	0.220	5.500	1.00270
Benzyl alcohol	0.5 cc.	0.05	0.025	0.625	0.11394
" "	0.5 "	0.50	0.080	2.000	0.36462
" "	0.5 "	1.00	0.140	3.500	0.63808
" "	0.5 "	2.00	0.175	4.375	0.79760
" "	0.5 "	4.00	0.190	4.750	0.86597
" "	0.5 "	8.00	0.200	5.000	0.91155
" "	0.5 "	16.00	0.210	5.250	0.95712
" "	0.5 "	32.00	0.225	5.625	1.02549

extract alone. This in itself is doubtless an important consideration since in the plant only small amounts of nitrate are normally present in the cells, and hence the reduction must take place at great dilution. The effect of larger amounts of sodium nitrate on the reduction is similar to that of salts on other biochemical processes. It is well known that at great concentrations even neutral salts exert a toxic action on all changes of this character.

On the Effect of Increasing the Quantity of the Potato Extract on the Reduction in the Presence of an Accelerator.

That the amount of nitrite formed increases with the quantity of the potato extract, when the latter is acting in the presence of formic or benzoic aldehyde, may be seen from the experiments recorded in Table IX. In this series of experiments the increase in the quantity of nitrite formed seems to be greater than could be expected from the increase in the amount of potato extract. Whether this is really the case or due to some slight error, which up to this time we have been unable to detect, can only be determined by still further observations along this line.

On the Influence of the Mass of the Accelerator on the Reduction.

It seemed desirable to determine the influence of the mass of the accelerator on the rate of reduction. Formic aldehyde was the accelerator tried, partly for the reason of its activity, and partly because of the fact that it is readily soluble in water. The data relative to the experiments, together with the quantities of nitrite produced in each case, are given in Table X.

Corresponding controls showed none or only traces of nitrite. The solutions in the tubes to which formaldehyde of greater strength than N/2 had been added were diluted before they were tested for nitrite, as solutions containing such comparatively large quantities of formaldehyde were found to interfere with the nitrite test.

It will be observed that the first effect of increasing the quantity of formic aldehyde is to increase the amount of ni-

Table IX.

Amount of potato extract varied as indicated in column 3 of table; 2 cc. of 35 per cent solution of potassium nitrate; the whole made up to a total volume of 13 cc. with distilled water.

Time, one hour. **Temperature, 40° C.**

Accelerator.	Amount of accelerator.	Amount of potato extract in cc.	Nitrite in 2 cc. expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in milligrams.
Formaldehyde	1 cc. N/5 solution	1	0.005	0.032	0.00717
"	1 " N/5 "	2	0.010	0.065	0.01456
"	1 " N/5 "	3	0.025	0.162	0.03630
"	1 " N/5 "	4	0.035	0.227	0.05087
"	1 " N/5 "	5	0.045	0.292	0.06544
"	1 " N/5 "	6	0.060	0.390	0.08734
"	1 " N/5 "	7	0.075	0.487	0.10913
"	1 " N/5 "	8	0.090	0.585	0.13109
"	1 " N/5 "	9	0.110	0.715	0.16023
"	1 " N/5 "	10	0.120	0.780	0.17480
Benzaldehyde	0.1 cc.	1	0.001	0.006	0.00134
"	0.1 "	2	0.005	0.032	0.00717
"	0.1 "	3	0.015	0.097	0.02174
"	0.1 "	4	0.035	0.227	0.05087
"	0.1 "	5	0.050	0.325	0.07283
"	0.1 "	6	0.120	0.780	0.17480
"	0.1 "	7	0.150	0.975	0.21850
"	0.1 "	8	0.200	1.300	0.29133
"	0.1 "	9	0.250	1.625	0.36416
"	0.1 "	10	0.300	1.950	0.43700

trite produced. This reaches a maximum with N/5 formic aldehyde, however, after which any increase in the amount of the aldehyde causes a diminution in the quantity of nitrite formed. It would seem, therefore, from these results that at greater concentration formic aldehyde is poisonous to the reducing substance or substances in the potato, which effect these changes.

Table X.

Ten cc. potato extract; 2 cc. of 35 per cent solution of potassium nitrate; 1 cc. of solution containing the indicated amount of formic aldehyde.

Time, one hour. Temperature, 40° C.

Accelerator.	Amount of formic aldehyde.	Nitrite in a cc. expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in milligrams.
Formaldehyde	1 cc. N/50 solution	0.030	0.195	0.04370
"	1 " N/10 "	0.230	1.495	0.33500
"	1 " N/5 "	0.250	1.625	0.36416
"	1 " N/2 "	0.160	1.040	0.23306
"	normal "	0.080	0.520	0.11653
"	1 cc. N/2 "	0.050	0.325	0.07283
"	1 " N/5 "	0.020	0.130	0.02913
"	1 " N/10 "	0.010	0.065	0.01457

On the Effect of Acids on the Reduction of Nitrates by Potato Extract in the Presence of an Accelerator.

The reduction of nitrates by the extract of the potato seems to take place best in a slightly acid medium. This is shown by the fact that even by simply neutralizing the very small amount of acid normally present in an active potato extract with alkali or lime-water its power to reduce a nitrate to nitrite is completely inhibited. It therefore occurred to us that it would be interesting to try the effect of acids on the progress of the reduction in the presence of an accelerator. This was tried, therefore, using a 4 per cent solution of formic aldehyde as an accelerator and N/10 solutions of the following

acids: hydrochloric, sulphuric, benzenesulphonic, *p*-nitrotoluenesulphonic, oxalic, formic, acetic, and butyric. The necessary data and results obtained are given in Table XI.

None of the controls contained any nitrite.

It will be observed that as a class acids exercise an inhibiting action on the progress of the reduction in the presence of an accelerator, the degree of inhibition depending upon the quantity of acid present and also on its strength, so that were we to arrange these acids with respect to their inhibitory effect on the change under consideration we would obtain the following: hydrochloric = sulphuric = benzenesulphonic = *p*-nitrotoluenesulphonic > oxalic > formic > acetic > butyric. It has been found further that while large amounts of the weaker acids inhibit the reduction, in smaller quantities they considerably accelerate it, and even the stronger acids, such as hydrochloric, etc., seem to accelerate the reduction, if present in very small amounts. We observe therefore that, so far as the action of acids on the change here under investigation is concerned, the effect produced is similar to that which they exercise on zymotic processes in general.

The Behavior of Other Plant Extracts towards Nitrates.

A priori, there is no reason for supposing that the power to reduce nitrates is confined exclusively to the potato tuber. With the view of learning something further as to the occurrence and distribution of such reducing substances in the vegetable kingdom, aqueous extracts of the plants enumerated in Table XII. were prepared and tried.

It will be observed, therefore, that of the plants thus far examined, the egg plant and the potato, tuber and sprouts, are the only ones from which we have been able to prepare extracts capable of reducing nitrates to nitrites. In this connection it should be observed that since this investigation was first undertaken, Pozzi-Escot[1] found that aqueous extracts of the stems of the burdock had the power to reduce nitrates to nitrites and ammonia. He concludes that enzymes which reduce nitrates are very abundant in actively growing plants,

[1] This JOURNAL, **29**, 539.

Table XI.

Ten cc. potato extract; 2 cc. 35 per cent potassium nitrate; 1 cc. 4 per cent formaldehyde; 1 cc. water, or solution containing the indicated amount of acid.

Time, one hour. Temperature, 40° C.

Name of acid.	Amount of acid.	Nitrite in 2 cc. expressed in cc. of standard.	Total nitrite expressed in cc. of standard.	Amount of nitrite in milligrams.
None	None	0.100	0.700	0.15687
Butyric	1 cc.	0.250	1.750	0.39217
Acetic	1 "	0.220	1.540	0.34511
Oxalic	1 "	0.185	1.295	0.29020
Formic	1 "	0.180	1.250	0.28236
Sulphuric	1 "	0.150	1.050	0.23530
Hydrochloric	1 "	0.140	0.980	0.21961
Benzenesulphonic	1 "	0.120	0.840	0.18824
p-Nitrotoluenesulphonic	1 "	0.110	0.770	0.17255
Butyric	2 "	0.170	1.190	0.26667
Acetic	2 "	0.140	0.980	0.21961
Oxalic	2 "	0.040	0.280	0.06274
Formic	2 "	0.050	0.350	0.07283
Sulphuric	2 "	0.000	0.000	0.00000
Hydrochloric	2 "	0.000	0.000	0.00000
Benzenesulphonic	2 "	0.000	0.000	0.00000
p-Nitrotoluenesulphonic	2 "	0.000	0.000	0.00000
Butyric	5 "	0.150	1.050	0.23530
Acetic	5 "	0.120	0.840	0.18824
Oxalic	5 "	0.000	0.000	0.00000
Formic	5 "	0.000	0.000	0.00000
Sulphuric	5 "	0.000	0.000	0.00000
Hydrochloric	5 "	0.000	0.000	0.00000
Benzenesulphonic	5 "	0.000	0.000	0.00000
p-Nitrotoluenesulphonic	5 "	0.000	0.000	0.00000

Table XII.

Ten cc. of the extract; 2 cc. of a 50 per cent solution of sodium nitrate; 0.5 cc. of toluene as antiseptic.

Time, one hour. Temperature, 40° C.

Name of plant.	Part of plant tested.	Amount of nitrite in millig'ms.
Potato[1] (*Solanum tuberosum*)	Tuber	0.10072
Egg plant (*Solanum melongena*)	Fruit	0.08195
Potato (*Solanum tuberosum*)	Sprouts, etiolated	0.02370
Artichoke (*Helianthus tuberosus*)	Tuber	None
Broom rape (*Orobanche ramosa*)	Entire plant	"
Radish (*Raphanus sativus*)	Root	"
Onion (*Allium Cepa*)	Bulb	"
Soy bean (*Glycine hispida*)	Leaves	"
Sweet potato (*Ipomoea batatas*)	Tuber	"
Apple (*Pyrus malus*)	Fruit	"
Banana (*Musa sapientum*)	"	"
Dahlia (*Dahlia variabilis*)	Tuber	"
Corn (*Zea mays*)	Unripe grain	"
Cinnamon vine (*Dioscorea divaricata*)	Axillary tuber	"
Coprinus atramentarius	Entire fungus	"
Wheat (*Triticum vulgare*)	Seedling	"
Buckwheat (*Fagopyrum esculentum*	"	"
Pea (*Pisum sativum*)	"	"
Barley (*Hordeum vulgare*)	"	"
Oats (*Avena sativa*)	"	"
Rye (*Secale cereale*)	"	"

especially at the end of a warm day. We have not yet had the opportunity to confirm this interesting and important observation. In connection with our failure to obtain extracts capable of reducing nitrates, it must be borne in mind that originally we experienced considerable difficulty in demonstrating this property satisfactorily even in the case of the potato, so that too much reliance cannot be placed in the negative results furnished by most of the plants examined. It may be that under slightly different conditions many, if not all of these, will still be found capable of reducing nitrates to nitrites. In this connection it has been found that the macera-

[1] For sake of comparison the potato is included.

ted tissue of the potato sprout reduces nitrates better than the aqueous extract thereof, indicating that the reducing substances bringing about this change are only slowly dissolved out of the protoplasm. This fact should also be taken into account in the interpretation of our results with other plants, and still further work on this phase of the problem is necessary.

Summary and Discussion of Results.

To sum up briefly the results of this part of the investigation, it has been found that the potato and certain other plants contain a reducing substance or substances capable of effecting the reduction of nitrates to nitrites. It has been found further that the reduction takes place most rapidly at 40° to 45° C., that it is augmented by an increase in the amount of nitrate present and also by an increase in the quantity of the reducing extract, that it is inhibited by certain poisons and acids and greatly accelerated by certain other substances, especially by aldehydes and alcohols, and that plant extracts capable of effecting this reduction completely lose this property when heated to boiling. It has also been found that the reduction in the presence of accelerators is subject to such conditions as modify or alter the reduction of the nitrate by the potato extract alone. In other words, it would seem that the effect of the accelerator is simply to increase the activity of the extract, without fundamentally changing the course of the reaction. Finally, as has already been pointed out above, a remarkable parallelism exists between the change under consideration and those brought about by the soluble ferments. In this connection the question suggests itself, how far is this a real analogy? While we are not yet in a position to answer this question satisfactorily, it should be observed that there has been a sort of loose tendency on the part of certain observers to put into the class of soluble ferments or enzymes all substances of animal or vegetable origin, which are destroyed in their aqueous solution by boiling, and which are more or less sensitive to the action of poisons. In consequence of this, an important property of the soluble ferment, namely, its

power to bring about chemical changes, in which the quantities of material changed are relatively very great and altogether disproportionate to the substance effecting the reaction, has apparently been lost sight of. Thus, for example, in biochemical oxidations and reductions there are many observations recorded which would indicate that the so-called oxidases and reductases are not enzymes in the strict sense of this definition. In fact, nearly all that is known concerning them goes to show that they enter only once into reaction, with the result that they themselves become so materially modified that they cannot act over again. Doubtless the fact that they are being continuously elaborated by the animal or plant has had a good deal to do with confusing them with the true enzymes. On the other hand, if with Berthelot[1] we look upon such an oxidizable substance as phosphorus as the prototype of these substances, we more nearly describe what they really can do, since phosphorus, whether acting as a reducing agent or a carrier of oxygen, is, likewise, limited in the oxidation and reduction which it can effect, the ultimate product of such reactions being phosphoric acid, a substance entirely incapable of inducing such changes. In this connection it should also be observed that while the number of oxygen carriers acting as true catalyzers in the sense that they are regenerated at the end of the chemical cycle, like platinum black and nitric oxide, is very limited, the number of other oxygen carriers is very large. In fact, if we admit the correctness of Engler's views of autoxidation, it follows that there are as many oxygen carriers as there are autoxidizable substances. Therefore, provisionally at least, we are inclined to put the reducing principles of the potato in the class of oxidizable substances, and to regard them as capable of reducing limited quantities of nitrate. At the same time we desire to call the attention of chemists to the necessity of finding some term other than fermentation whereby to distinguish such biochemical processes as the one here under consideration.

The Action of Accelerators on the Reduction—Traube's Views.

From a purely chemical standpoint the accelerating action

[1] Berthelot: Chimie Vegetale et Agricole, 3, 183.

of certain substances, such as aldehydes and alcohols, on the reduction of nitrates by the potato extract is perhaps the most interesting feature of our results.

In this connection it is interesting to note that Traube in his Theorie der Fermentwirkungen called attention to certain similar changes. According to this observer the ferments possess the power to give up the oxygen removed from a second substance to a third, and just as the oxydases and ferments of putrefaction carry free oxygen to other substances, so the reducing ferments carry to other oxidizable substances the oxygen taken up as the result of reduction. In this connection he cites the fact that yeast reduces indigosulphuric acid better in the presence of sugar than without it. Here again this theory should be extended so as to include unstable substances resulting from vital activity, but not true ferments. It is conceivable, for example, that the reducing substances in the potato remove oxygen from the nitrate and in turn oxidize the aldehyde or other accelerator. In so doing, the original reducing substance might of course be regenerated as such, in which event the substance in question could once more enter into reaction and thereby ultimately reduce very large amounts of nitrate. It seems more probable, however, that a reducing substance sufficiently powerful to remove oxygen from potassium nitrate would retain a part at least of the oxygen thus removed, at the same time that it gave up a portion of it to the accelerator. The result would be that the reducing substance would be gradually oxidized, and hence unable to reduce disproportionately large amounts of nitrate.

As to the mechanism of the action of the accelerator but little is known at present. It may be that the acceleration is due to the fact that the affinity of two reducing substances for the oxygen of the nitrate, *viz.*, that of the reducing substance in the plant extract and that of the aldehyde or alcohol, is greater than the affinity of either alone. This would account for the increased production of nitrite resulting from the addition of the accelerator. Or it may be, as Traube believes, that the reducing substance of the plant alternately takes oxygen from the nitrate and gives it up to the accelerator. In

this event also the effect of such third substances would be to accelerate the change.

A third explanation suggests itself. The reduction of the nitrate by the reducing substance in the potato might also be brought about by the decomposition of water, the nascent hydrogen thus produced reducing the nitrate. The presence of a second reducing substance might then accelerate the reaction by virtue of its affinity for oxygen. This view derives considerable support from the fact that under anaerobic conditions certain of the unorganized ferments, such as the butyric acid ferment and other putrefying bacteria, can cause the evolution of hydrogen from fermenting liquids. It also derives support from the early work of Pollacci[1] on the evolution of hydrogen sulphide from grapes and other plants that have been sprinkled with sulphur, and also from the later work of de Rey-Pailhade[2] and Pozzi-Escot[3] on the formation of hydrogen sulphide by the action of philothion and the reductases on sulphur. According to the first of these observers, the production of hydrogen sulphide is due to the action of nascent hydrogen resulting from the decomposition of water. Traube also inclined to this view of the mode of action of the reducing ferments. It also derives support from the following considerations. In the second part of this paper it will be shown that a platinum zinc alloy has the power of reducing a nitrate to nitrite, and that this power is also greatly increased by certain accelerators, notably formaldehyde. Now it has been shown by Gladstone and Tribe[4] that the action of metallic couples in reducing nitrates is due primarily to the electrolytic decomposition of water by the metallic couple, the nascent hydrogen produced by the electrolysis reducing the nitrate, the oxygen combining with the metal. The explanation of de Rey-Pailhade[5] regarding the formation and action of the reducing substance which he has called philothion also involves the decomposition of water. Accord-

[1] Gazz. chim. ital., **3**, 451–460.

[2] Compt. rend., **106**, 1683–1684; **107**, 43–44, et seq.

[3] This JOURNAL, **29**, 517–563.

[4] J. Chem. Soc. (London), **33**, 150.

[5] Given by Pozzi-Escot in an article on the "Reducing Enzymes," This JOURNAL, **29**, 522.

ing to this author philothion is probably to be represented by the general formula RH_2, when this hydrogenizes sulphur or reacts with oxygen we have:

$$RH_2 + S = R + H_2S,$$

and

$$RH_2 + O = R + H_2O.$$

The substance represented by R, however, may according to this observer, regenerate philothion by the combined action of it and an oxidizable substance R′ on water. Thus:

$$R + H_2O + R' = RH_2 + R'O.$$

According to Pozzi-Escot[1] the reductases probably have an aldehyde nature in which case they probably effect the addition of hydrogen to such substances as sulphur by the decomposition of water. We observe therefore that all of these explanations have this in common, *viz.*, the idea that the hydrogen and oxygen necessary for these transformations are furnished by the decomposition of water.

A further interest attaches to our results on the action of aldehydes as accelerators in the reduction of nitrate by the potato extract. According to Gautier[2] the reduction of nitrates in the leaves of plants is accomplished by means of aldehydes. While our own results are not altogether in harmony with this view, it is interesting to note that the aldehydes do accelerate the reduction very greatly, and it is conceivable that in the plant normally the reduction of nitrate is effected not by any one reducing substance but by such a substance acting in the presence of an aldehyde, normally occurring in the cell. In this connection it should be observed that Curtius and Reinke[3] have obtained from the leaves of certain green plants a di- or tetrahydro aromatic aldehyde-alcohol to which they have assigned the formula

$$C_6H_8\begin{cases}CHO\\CH_2OH\end{cases} \quad \text{or} \quad C_6H_8\begin{cases}CHO\\CH_2OH\end{cases}.$$

In the light of our results there can be but little question that

[1] Given by Pozzi-Escot in an article on the "Reducing Enzymes," This JOURNAL, 29, 361.

[2] Lecons de Chimie Biologique, Ed. 2, p. 36.

[3] Ber. d. bot. Ges., 18, 201.

such a compound would at least assist materially in the reduction of nitrates by the growing plant.

On the Reduction of Nitrates by Metals in the Presence of Accelerators.

Several years ago Loew[1] called attention to the fact that when platinum black, glucose, and potassium nitrate are brought together under certain conditions, ammonia is formed. It will be observed that this case presents a certain analogy to the reduction of a nitrate by the potato extract in the presence of an accelerator, the extract taking the place of the platinum black in Loew's experiment. The analogy becomes all the more striking when it is borne in mind that in both instances aldehydes function as accelerators. It therefore occurred to us to test the conduct of platinum black towards a nitrate, both in the presence and absence of an accelerator.

The platinum black employed in our first series of experiments was prepared by precipitating the metal from its chloride by means of zinc dust. The precipitate was washed by decantation, first with hydrochloric acid and finally with distilled water. It was then transferred to a filter and washed with distilled water until free from chloride. Finally it was thoroughly dried at ordinary temperature.

Series 1.—Three tubes were prepared as follows: No. 1 contained 0.1 gram platinum, 10 cc. water, 1 cc. of a 4 per cent solution of formaldehyde, and 2 cc. of a 50 per cent solution of sodium nitrate. No. 2 differed from No. 1 only in that it contained 1 cc. water in place of the 1 cc. of formaldehyde in the latter. No. 3 differed from No. 1 only in that it contained no platinum.

The tubes were tightly corked and shaken, and then allowed to remain at 40° C. for seventeen hours. At the end of this time the contents of each tube was tested for nitrite, and the amount of nitrite determined in the usual manner. The following results were obtained:

[1] Ber. d. chem. Ges., 23, 675 (1890).

No. of experiment.	Total nitrite in milligrams.
1	2.37003
2	0.05925
3	0.00000

Series 2.—This differed from the above only in that the time allowed for the experiments was a little longer, namely, twenty-four hours. The following results were obtained:

No. of experiment.	Total nitrite in milligrams.
1	3.37729
2	0.07110
3	0.00000

Series 3.—This was exactly similar to the above, except that the time was increased to forty-eight hours. The following are the results obtained:

No. of experiment.	Total nitrite in milligrams.
1	4.26605
2	0.07703
3	0.00000

Series 4.—This differed from the former series in containing 1 cc. of 90 per cent alcohol as the accelerator in place of the 1 cc. of formaldehyde. After standing for five hours the following results were obtained:

No. of experiment.	Total nitrite in milligrams.
1	0.56288
2	0.01185
3	0.00000

Series 5.—In this series 1 cc. of N/10 formic acid was used as the accelerator in place of the formaldehyde or alcohol in the former experiments. A 35 per cent solution of potassium nitrate was also substituted for the 50 per cent solution of sodium nitrate used in the previous experiments. After standing for two hours at 40° C. the following results were obtained:

No. of experiment.	Total nitrite in milligrams.
1	0.17775
2	0.00091
3	0.00000

Other substances which were found to work quite well as

accelerators in the case of the potato, such as benzaldehyde, benzyl alcohol, and vanillin, were also tried with platinum in place of the potato extract, but the results obtained were very indefinite, certainly not so striking as in the cases just given. While the cause of this apparent difference in the effect of these accelerators towards the potato extract and platinum has not yet been investigated, it is not unlikely that the reason of the inactivity of these substances is due to their insolubility, and thus unlike the system obtained with the potato extract, these furnish systems, with two insoluble substances which therefore have little chance of coming in contact with one another.

It is evident from these results, therefore, that sodium and potassium nitrates are reduced to nitrite by platinum black, and that the amount of nitrite so produced is greatly increased (from 10 to 60 times) by the presence of certain third substances, such as ethyl alcohol, formaldehyde, and formic acid.

On the Effect of Heat on the Reducing Power of Platinum.

In this connection it occurred to us to determine the effect of heat on the activity of platinum black as a reducing agent. In order to test this point a specimen of platinum, prepared as indicated above, was divided into three portions. One portion was kept at ordinary temperature, a second was heated to about 300° C. for about twenty or thirty minutes, and a third was heated to redness for the same length of time. With these three specimens the following experiments were tried:

Series 6.—No. 1. 0.1 gram platinum, that was not heated, was brought together in a test-tube with 11 cc. of water and 2 cc. of 35 per cent solution of potassium nitrate. No. 2. Same as (1), except that it contained 1 cc. of a 4 per cent solution of formaldehyde in place of 1 cc. of water as in (1). No. 3. Same as (1), except that 0.1 gram platinum that had been heated to about 300° C. was used in place of platinum at ordinary temperature. No. 4. Same as (2), except that 0.1 gram of platinum (heated to about 300° C.) was employed. No. 5. Same as (1) and (3), except that 0.1 gram of platinum that had been heated to redness for half an hour was

employed. No. 6. Same as (2) and (4), except that 0.1 gram of the platinum (heated to redness) was used.

The tubes containing these several substances were allowed to stand at 40° C. for four hours, at the end of which time the quantity of nitrite in each was determined. The following results were obtained:

No. of experiment.	Total nitrite in milligrams.
1	0.01457
2	0.14567
3	0.00000
4	16.02315
5	0.00000
6	2.03931

These results are of interest, first as showing that formaldehyde greatly accelerates the reduction of nitrate by platinum, just as it was found to in the case of the potato extract. In fact, with the platinum that had been heated to about 300° C., and to red heat, its presence seemed to be essential for the reduction, since no nitrite was formed when it was not at hand. The most interesting fact brought to light by these experiments, however, is that heat increases the activity of platinum black when acting in the presence of an accelerator, and that as compared with the activity of the metal that had been kept at ordinary temperature, and that which had been heated to redness, that which had been heated to about 300° C. was by far the most active.

Another series of experiments was tried with some of the same platinum black as that employed in Series 6. In this series some of the metal that had been previously heated to 100° C. and 300° C. respectively for thirty minutes was compared with some that had been kept at ordinary temperature. Six tubes were prepared. Nos. 1, 2, 3, and 4 were similar in every respect to Nos. 1, 2, 3, and 4 in the first series of experiments. Nos. 5 and 6 differed from the corresponding ones in Series 6, in that they each contained 0.1 gram of the platinum black that had been heated to 100° C. instead of that which had been heated to redness. These tubes were kept at 40° C. for three hours. At the end of this time the amount of nitrite was determined in each with the following results:

Number of experiment.	Total nitrite in milligrams.
1	0.01165
2	0.11653
3	0.00000
4	5.82660
5	0.00291
6	0.18936

It will be observed that while the activity of the platinum is slightly increased by heating it to 100° C. for half an hour, previous to the experiment, it by no means compares in activity with that which had been heated to 300° C.

In view of this remarkable increase in the reducing powers of the metal brought about by heating it to 300° C. it occurred to us to study the conduct of platinum black, prepared by other methods, towards a nitrate in the presence of an accelerator. Accordingly, platinum black was prepared by the following methods: By Loew's[1] method, by Zdrawkowitch's[2] method, by reducing platinum chloride with metallic magnesium and washing with hydrochloric acid, by reduction of the chloride with Kahlbaum's pure zinc and washing with acid, and by heating ammonium chlorplatinate to dull redness. The activity of these several varieties of the metal was tested in the manner already described in the experiments with platinum black obtained by the reduction of the chloride with zinc dust, Series 6. Strange to say, none of these specimens possessed the activity of the metal obtained by precipitating with zinc dust, and certain of them gave not even the slightest indication of the formation of nitrite. That obtained from ammonium chlorplatinate and by Loew's method gave only slight traces of nitrite. This is all the more remarkable when we consider that Loew's platinum black is said to be a very active variety of the metal, and one specimen of this was found by us to readily oxidize aldehydes in the presence of air. It therefore occurred to us that possibly the active platinum black, which was obtained by precipitating the metal with zinc dust, and also with stick zinc, contained other substances, in spite of the fact that in

[1] Ber. d. chem. Ges., **23**, 289 (1890).
[2] Ann. Chem. (Liebig), **181**, 192.

every case it had been washed with hydrochloric acid, and in some cases with both hydrochloric and nitric acids. The amount of platinum in these several specimens of the metal that had been prepared by means of zinc was therefore determined by the usual method of converting into ammonium chlorplatinate and weighing as platinum. The following are the results of several of these determinations:

		Per cent. of platinum.
1	Platinum black, obtained by precipitating with zinc dust and washing with dilute hydrochloric acid.	85.5
2	Same as (1) but stood under nitric acid over night.	89.45
3	Prepared by precipitating with pure stick zinc, and washing with dilute hydrochloric acid.	90.46
4	Obtained by precipitating with filings of Kahlbaum's pure stick zinc and washing with acid.	86.42

The metallic impurity in these several specimens was found to consist chiefly of zinc. It seems therefore that it is impossible to remove all of the zinc from platinum black that has been prepared by precipitating with this metal, by washing with dilute acids. This naturally suggests that the platinum black thus obtained is really an alloy of zinc and platinum, and that it owes its power to reduce a nitrate to the zinc which it contains. The effect of heat in increasing the activity is none the less interesting, however, and the question still remains to be considered, why is the activity of this material so greatly increased by heating to 300° C.? While this question has not been satisfactorily answered as yet, it is interesting to note in this connection that Deville and Debray[1] have observed a curious change in the character of zinc alloys of certain of the platinum metals at 300° C., notably in the case of iridium. Some similar change then seems to occur in the platinum zinc alloy at this temperature. It has occurred to us that this change may consist in the lessening of the attraction of the zinc for the platinum in the alloy.

[1] Chem. Centrbl., 1882, 468; Compt. rend., 64, 1557–60.

This would satisfactorily explain the increased activity of the material that has been heated to this temperature, since it is known that zinc itself rapidly reduces nitrates. On the other hand, we know from the work of Gladstone and Tribe[1] that certain couples of metals, as the copper-zinc couple, also reduce nitrates and effect other reactions with great rapidity. It may be therefore that the platinum-zinc alloy is really a couple of this description, and that the effect of the heat is to cause an alteration in the couple, whereby it becomes more active. It is known for example, that the activity of the so-called dry copper-zinc couple in certain reactions depends a great deal on the manner of heating the two metals in its preparation. However, all these matters require further investigation. We hope in the future to study the effect of such reducing-agents as formic aldehyde on the reduction of nitrate by metallic couples of various kinds. In fact, some evidence of the accelerating action of formic aldehyde in the reduction of nitrates by metallic couples has already been obtained.

On the Influence of Accelerators on the Reduction of Nitrate by Zinc-Silver Alloys.

The results reached in our study of the reduction of potassium nitrate by platinum black led us to test the conduct of precipitated silver towards a solution of the nitrate. In our first experiments finely divided silver was obtained by precipitating a dilute solution of silver nitrate with zinc dust, washing the precipitate first with dilute nitric acid and finally with distilled water. Under these conditions, the silver was obtained in the form of a grayish white powder. On analysis, it was found to consist entirely of metallic silver. It was found to be entirely inactive towards potassium nitrate even in the presence of an accelerator. This, then, is in keeping with our results with pure platinum black. On the other hand, when a strip of zinc is immersed in a dilute solution of silver nitrate the silver is obtained in the form of a loosely adhering black or very dark gray coating on the surface of the

[1] J. Chem. Soc. (London), 33, 139, 151 (1878).

zinc. If this be removed from the surface of the zinc by constant stirring and washed with water, but not with acid, a dark grayish-black powder is obtained altogether different in appearance from the lighter silver-gray powder obtained by washing the precipitated silver with dilute nitric acid. On analysis, this dark powder was found to contain only 90.1 per cent. of metallic silver. This dark material therefore is in all probability a true alloy of zinc and silver analogous in composition to the platinum black obtained by precipitating platinum chloride with zinc dust. On adding acid to this dark-colored material it becomes grayish white in appearance and altogether similar to pure silver in finely divided condition. Ludtke[1] seems to regard this black silver sponge obtained by reducing silver nitrate with zinc as a distinct modification of metallic silver. He also has observed that acids transform it into the gray variety, and that this change is accompanied by an increase in the electrical conductivity of the material. His results can be better explained, however, on the supposition that the black sponge is an alloy of zinc and silver. Like the platinum-zinc alloy, this black silver sponge has been found to be an exceedingly active denitrifying material, especially when allowed to act upon the nitrate in the presence of certain other substances, such as formaldehyde.

That such is the case may be seen from the following experiments:

In order to determine the reducing power of this silver-zinc alloy three series of experiments were tried. In Series A three tubes were prepared. No. 1 contained 0.1 gram of the silver-zinc alloy, 10 cc. of water, 1 cc. 4 per cent formaldehyde, and 2 cc. of a 35 per cent solution of potassium nitrate. No. 2 was same as No. 1, except that it contained 1 cc. of water instead of 1 cc. of formaldehyde. No. 3 was the same as No. 1, except that it contained no metal. These tubes were kept at 40° C. for two hours.

Series B.—Nos. 1, 2, and 3 same as 1, 2, 3 in Series A, except that the tubes were heated to 40° C. for four hours.

[1] Ann. Phys. Chem. [2], **30**, 678, 695.

Series C.—Nos. 1, 2, and 3 same as corresponding experiments in Series A, except that the tubes were kept in the bath at 40° C. for fifteen hours.

At the end of these several intervals respectively, the several amounts of nitrite were determined colorimetrically, with the following results:

Series.	No. of experiment.	Total nitrite in milligrams.
A	1	7.86591
"	2	0.01456
"	3	0.00000
B	1	11.65320
"	2	0.02000
"	3	0.00000
C	1	21.84975
"	2	0.15000
"	3	0.00000

It is evident from these results that the amount of nitrite formed by the action of the silver-zinc alloy on potassium nitrate is greatly increased by the presence of small amounts of formaldehyde. Indeed, of all the substances thus far tried, formaldehyde has been found to be by far the most active. Whether, as was found in the case of the platinum-zinc alloy, heat increases the activity of the silver-zinc alloy still remains to be determined.

Up to the present only one phase of the process of denitrification, namely, the formation of nitrite by the several agencies herein mentioned, has engaged our attention. The formation of ammonia is doubtless also an important phase of this process. It is our intention to undertake an experimental study of this phase of the reduction as soon as time will permit, and also to investigate more thoroughly the effect of various reducing agents on the reduction of nitrates by various metallic couples.

The expenses of this investigation have been paid for out of a grant allowed to Mr. Elvove by the Carnegie Institution of Washington, for which assistance due acknowledgment is hereby made.

STATE COLLEGE OF KENTUCKY,
LEXINGTON, KENTUCKY,
October, 1903.

THE REACTION BETWEEN UNSATURATED COMPOUNDS AND ORGANIC MAGNESIUM COMPOUNDS.

I. REACTIONS OF UNSATURATED ALDEHYDES AND UNSATURATED KETONES.

By E. P. Kohler.

In a paper on "diphenylstyrylcarbinol"[1] I described the reaction between benzalacetophenone and phenylmagnesium bromide. The simplest substance obtained by this reaction was regarded as a tertiary alcohol represented by the formula $C_6H_5CH:CHC(OH)(C_6H_5)_2$. This view was based on the following facts:

1. The method of preparation would be expected to give a substance represented by the formula proposed. With saturated ketones Grignard's reaction gives tertiary alcohols, and, in all the cases that have been described, the products obtained from 1,2-unsaturated compounds are of the same kind as those obtained from the corresponding saturated compounds.[2]

2. The substance readily reacts with phosphorus pentachloride, the result being the elimination of an oxygen atom and a hydrogen atom, and the introduction of one chlorine atom.

3. The substance decolorizes a solution of bromine in carbon bisulphide in the cold. The product, which contains only 1 atom of bromine, was supposed to be formed by addition of bromine and subsequent loss of hydrobromic acid.

4. When oxidized with potassium permanganate the substance breaks down into benzophenone, benzoic acid, and carbon dioxide. This result was interpreted as follows:

$$\text{I. } C_6H_5CH:CHCOH(C_6H_5)_2 + 4O = C_6H_5CO_2H + (C_6H_5)_2COHCO_2H;$$

$$\text{II. } (C_6H_5)_2COHCO_2H + O = (C_6H_5)_2CO + H_2O + CO_2.$$

These results agreed with the proposed formula, but attention was drawn to the unexpected inertness of the hydroxyl

[1] This Journal, 29, 352.

[2] Ann. chim. phys., [7], 24, 465-478; Ber. d. chem. Ges., 35, 3188.

group in the carbinol and of the chlorine atom in the chloride. The substance was made with the expectation that the hydroxyl group would be as reactive as that of triphenylcarbinol and its behavior seemed inexplicable. Attention was also drawn to the fact that the substance forms no ether with alcohols and no esters with acid chlorides or acid anhydrides. This was attributed to the influence of the groups in combination with the tertiary carbon atom, since similar difficulties are met within the preparation of esters of triphenylcarbinol. Since the publication of the paper I have found that all these peculiarities are explained by the fact that the substance is not a tertiary alcohol at all, but an isomeric ketone represented by the formula

$$(C_6H_5)_2CHCH_2COC_6H_5.$$

This formula is established by the following facts:

1. The substance forms a phenylhydrazone and an oxime.

2. By Beckmann's rearrangement the oxime passes into an acid anilide which, on hydrolysis, gives β,β-diphenylpropionic acid:

$$\text{I.}\quad (C_6H_5)_2CHCH_2C(NOH)C_6H_5 \longrightarrow (C_6H_5)_2CHCH_2CONHC_6H_5;$$

$$\text{II.}\quad (C_6H_5)_2CHCH_2CONHC_6H_5 + H_2O = (C_6H_5)_2CHCH_2CO_2H + C_6H_5NH_2.$$

3. The substance can be made by condensing benzene with benzalacetophenone in the presence of concentrated sulphuric acid:

$$C_6H_5CH:CHCOC_6H_5 + C_6H_6 = (C_6H_5)_2CHCH_2COC_6H_5.$$

4. The substance reacts with phenylmagnesium bromide to form the same tertiary alcohol that is obtained by the action of phenylmagnesium bromide on the methyl ester of β,β-diphenylpropionic acid:

I. $(C_6H_5)_2CHCH_2CO.OCH_3 + 2C_6H_5MgBr = (C_6H_5)_2CHCH_2C(C_6H_5)_2OMgBr + MgOCH_3Br$;

II. $(C_6H_5)_2CHCH_2COC_6H_5 + C_6H_5MgBr = (C_6H_5)_2CHCH_2C(C_6H_5)_2OMgBr$.

These results prove that the earlier formula is wrong and that the substance is β,β-diphenylpropiophenone. The facts used in support of the earlier formula are explained as follows:

1. The product of the reaction with phosphorus pentachloride is triphenylchlorpropylene,

$$(C_6H_5)_2CHCH = CClC_6H_5.$$

2. The action of bromine is direct substitution; the product, as will be shown below, is α-brom-β,β-diphenylpropiophenone, $(C_6H_5)_2CHCHBrCOC_6H_5$.

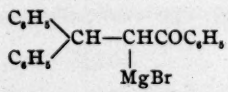
3. The oxidation with permanganate is represented by the equation:

$$(C_6H_5)_2CHCH_2COC_6H_5 + 5O = (C_6H_5)_2CO + CO_2 + C_6H_5CO_2H + H_2O.$$

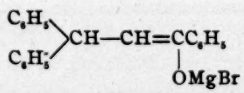
4. The dimolecular reduction-product is the pinacone

$$\begin{array}{l}(C_6H_5)_2CHCH_2COHC_6H_5\\ \quad\quad\quad\quad\quad\quad\quad\quad|\\ (C_6H_5)_2CHCH_2COHC_6H_5\end{array}.$$

The formation of diphenylpropiophenone from benzalacetophenone illustrates a new kind of reaction of organic magnesium compounds. The only magnesium compounds that, when decomposed with water, would give this ketone, are represented by the formulas:



I.



II.

It follows that the phenylmagnesium bromide, in this case, does not combine with the carbonyl group as it does in the

case of saturated ketones; but, either with the unsaturated carbon atoms of the chain—giving Formula I.—or at the extremities of the conjugated system, C=C—C=C, giving Formula II. The relation between these two formulas is exactly the same as that between the formulas used to represent the metallic derivatives of substances like acetoacetic ester. The properties of the magnesium compounds are essentially the same as those of the metallic derivatives referred to, so that this reaction makes it possible to get, indirectly, a number of reactive metallic derivatives of substances that do not have sufficiently mobile hydrogen atoms to form such derivatives by direct replacement.

The facts at present available are not sufficient to make it possible to decide, conclusively, between the two formulas, but I believe that Formula II. gives the simplest explanation of all the reactions that have been studied. When the magnesium compound is treated with water it yields diphenylpropiophenone, and when it is treated with bromine it yields α-bromdiphenylpropiophenone. These results are explained equally well by both formulas. The substance also reacts with acid chlorides. A substance with Formula I., which represents a compound of the type RMgBr, should give, with acid chlorides, a magnesium derivative of a tertiary alcohol. A substance with Formula II., on the other hand, should give an ester and a magnesium salt. The substance in question reacts with benzoyl chloride according to the equation:

$$(C_6H_5)_2CH{-}CH{=}C(OMgBr){-}C_6H_5 + C_6H_5COCl = (C_6H_5)_2CH{-}CH{=}C(OCOC_6H_5){-}C_6H_5 + MgClBr.$$

The reaction was carried out in a freezing-mixture and the amount of ester obtained was 98 per cent of the theoretical yield. The formation of this ester can, of course, be ex-

plained on the assumption that the structure of the magnesium compound is represented by Formula I.; but as there is not the slightest evidence in favor of this formula, I prefer Formula II., which gives the result as one of direct replacement.

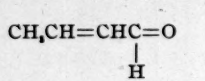
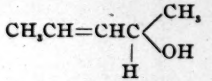
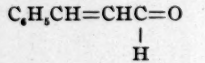
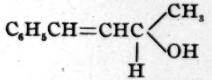
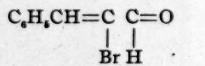
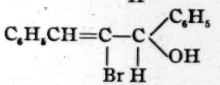
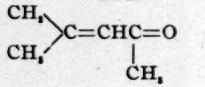
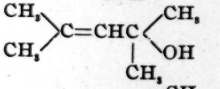
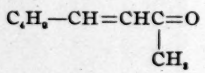
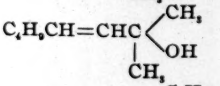
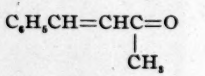
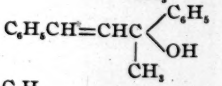
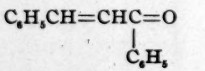
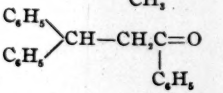
Moreover, a magnesium compound with the structure represented by Formula I. should react with saturated aldehydes and ketones, and with esters of saturated acids. I have tried five magnesium compounds of this type with seven different ketones and six esters, and have failed to get a reaction in a single case. These magnesium compounds readily combine, however, with unsaturated aldehydes, unsaturated ketones, and unsaturated esters, just as the metallic derivatives of acetoacetic ester combine with a variety of unsaturated compounds. The study of these addition-reactions has not been completed, hence the results will be reserved for a later paper.

A number of experiments have been made for the purpose of ascertaining to what extent α,β-unsaturated compounds combine with organic magnesium compounds in the manner just described in the case of benzalacetophenone. The work in this direction is not nearly complete, but the results already obtained indicate some of the factors that determine the course of the reaction between these substances.

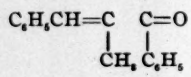
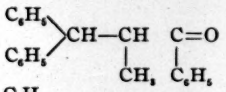
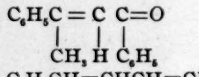
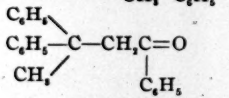
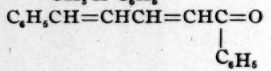
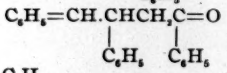
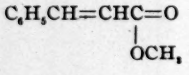
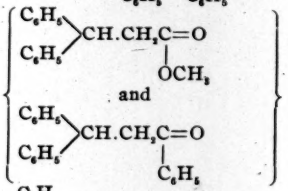
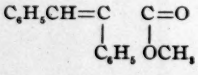
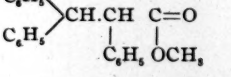
The result is, in all probability, independent of the nature of the magnesium compound, since it has been found that methylmagnesium iodide, isopropylmagnesium bromide, phenylmagnesium bromide, benzylmagnesium chloride, and naphthylmagnesium bromide, give the same kind of reaction with a given unsaturated compound.

The result depends entirely upon the nature of the unsaturated compound. The following table gives the results that have been obtained with a selected list of typical unsaturated compounds. A comparison of these results with those obtained in other addition-reactions of the same substances will be given in a subsequent paper.

Summary of Reaction between α,β-Unsaturated Compounds and Organic Magnesium Compounds.

	CH_3MgI		Grignard: Ann. Chim. Phys., [7], **24**, 465.
	CH_3MgI		Sand and Singer: Ber. d. chem. Ges., **35**, 3185.
	C_6H_5MgBr		This paper.
	CH_3MgI		Grignard: *Ibid.*, **35**, 477.
	CH_3MgI		Grignard: *Ibid.*, **35**, 478.
	C_6H_5MgBr		This paper.
	CH_3MgI		" "

Summary of Reaction between α,β-Unsaturated Compounds and Organic Magnesium Compounds (Continued).

	C_6H_5MgBr		" "
	C_6H_5MgBr		" "
	C_6H_5MgBr		Unpublished results. This laboratory.
	C_6H_5MgBr		" "
	C_6H_5MgBr		" "

EXPERIMENTAL PART.

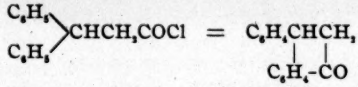
Method of Procedure. When a solution of an unsaturated ketone is gradually added to an ethereal solution of an organic magnesium compound, an amorphous precipitate invariably separates at once. At first this precipitate redissolves rapidly, but as the reaction proceeds the solution takes place more and more slowly until, finally, the precipitate no longer disappears when the liquid is shaken vigorously. Up to this point only one substance is formed. If more ketone is added the result is a mixture from which it is difficult to separate pure substances. The precipitate usually redissolves until the solution contains approximately half a molecule of ketone for every molecule of the magnesium compound; but somewhat more ketone can be used at low than at high temperatures.

In all the experiments with ketones the reaction was carried out as follows: An ethereal solution of the ketone was added, drop by drop, to a similar solution of the magnesium compound that was kept below —10°, until a permanent precipitate formed in the liquid. The mixture was then removed from the freezing-mixture and allowed to remain at the temperature of the laboratory for several hours, after which it was carefully decomposed with ice and the magnesium salts dissolved in iced hydrochloric acid. The organic product was dissolved in ether, the ethereal solution dried over calcium chloride, and the ether evaporated in a rapid current of dry air. The solids that separated were purified by recrystallization from alcohol.

I. Reactions with Benzalacetophenone.

1. Benzalacetophenone and Phenylmagnesium Bromide: Diphenylpropiophenone, $(C_6H_5)_2CHCH_2COC_6H_5$.—The analysis and properties of this substance were published in a previous paper. I tried to prepare it from diphenylpropionic acid and benzene by the Friedel and Crafts reaction. Five grams of aluminium chloride were added to a solution of 20 grams of diphenylpropionyl chloride in 50 grams of benzene that was kept cool with running water. The substances reacted in the

cold and the reaction was complete in a very short time. The product, isolated in the usual way, was a solid melting at 78°. Analysis and properties showed that the substance is keto-phenyldihydroindene, formed according to the equation :



The yield was 13 grams. While the ketone cannot be made by the Friedel and Crafts reaction, it is easily obtained by direct condensation of benzalacetophenone and benzene. A solution of 10 grams of benzalacetophenone in 100 grams of benzene was treated with 10 grams of concentrated sulphuric acid. After shaking the mixture mechanically for six days, the benzene layer was separated, washed with water, and distilled with steam. The residue—six grams—was almost pure diphenylpropiophenone.

The phenylhydrazone of diphenylpropiophenone was obtained by boiling an alcoholic solution of the ketone with a slight excess of phenylhydrazine for eight hours. The product was purified by recrystallization from alcohol. It crystallizes in pale yellow needles that melt at 137°. The analyses gave the following results :

I. 0.1793 gram substance gave 0.5625 gram CO_2 and 0.1045 gram H_2O.

II. 0.4868 gram substance gave 32.2 cc. N at 20° and 756 mm.

	Calculated for $C_{27}H_{24}N_2$.	Found. I.	Found. II.
C	86.11	85.97	....
H	6.35	6.48	....
N	7.45	....	7.49

The oxime was made by boiling an alcoholic solution of 10 grams of the ketone, 10 grams of hydroxylamine hydrochloride, and 18 grams of potassium hydroxide for four hours. The mixture was poured into cold water and the precipitated oxime recrystallized from aqueous alcohol. The oxime crystallizes well in large, lustrous needles. The melting-point is at 131°. An analysis gave the following results :

I. 0.1798 gram substance gave 0.5510 gram CO_2 and 0.1061 gram H_2O.

II. 0.6101 gram substance gave 26.3 cc. N at 21° and 756 mm.

	Calculated for $C_{21}H_{19}ON$.	Found. I.	II.
C	83.72	83.56	....
H	6.31	6.55	....
N	4.65	....	4.93

Diphenylpropionanilide was obtained from the oxime of diphenylpropiophenone by Beckmann's rearrangement. For this purpose 5 grams of phosphorus pentachloride were added to an equal weight of the oxime dissolved in absolute ether. When the reaction was over, the ether was removed by distillation and the oily residue boiled with water until it had solidified completely. The anilide was purified by recrystallization from aqueous alcohol. It crystallizes in needles melting at 167°. It is readily soluble in alcohol and ether, sparingly soluble in ligroin.

0.1581 gram substance gave 0.4846 gram CO_2 and 0.0911 gram H_2O.

	Calculated for $C_{21}H_{19}ON$.	Found.
C	83.72	83.59
H	6.31	6.40

The anilide was hydrolyzed by heating it with hydrochloric acid in a sealed tube at 150°. The resulting acid was recrystallized from weak alcohol. It melted at 147°. It was compared with a specimen of β,β-diphenylpropionic acid prepared by condensing benzene with cinnamic acid. A mixture of the two melted sharply at 147°—the melting-point of pure diphenylpropionic acid.

Diphenylethyldiphenylcarbinol, $(C_6H_5)_2CHCH_2COH(C_6H_5)_2$.—When benzalacetophenone is treated with phenylmagnesium bromide the reaction always stops after the introduction of one phenyl group. To determine whether this is due to the properties of the resulting magnesium compound or to the combination of groups present in the substance, the ketone

obtained by decomposing the magnesium compound with water was treated with phenylmagnesium bromide in the usual way. The product has almost exactly the same melting-point as the ketone used in the reaction. A mixture of the two, however, begins to melt 20° lower and the analysis shows that it is a different substance.

0.1501 gram substance gave 0.4924 gram CO_2 and 0.0896 gram H_2O.

	Calculated for $C_{27}H_{24}O$.	Found.
C	89.01	89.4
H	6.59	6.63

The carbinol is readily soluble in alcohol and ether, moderately soluble in ligroin. It crystallizes in needles that melt at 95° to 96°. The same substance is obtained by treating the methyl ester of diphenylpropionic acid with phenylmagnesium bromide. This result proves that it is the tertiary alcohol represented above. The alcohol cannot be made by treating the magnesium compound, that gives diphenylpropiophenone when decomposed with water, with an excess of phenylmagnesium bromide. This result would be expected if, as seems probable, the structure of the magnesium compound is different from that of the ketone, namely,

$$(C_6H_5)_2CH.CH=C\begin{matrix}OMgBr\\ C_6H_5\end{matrix}.$$

It may, however, be due to the influence of the α-substituent in a compound of the structure

$$(C_6H_5)_2CH.\underset{\displaystyle MgBr}{\underset{|}{C}}HCOC_6H_5,$$

since results obtained with unsaturated acids show that some α-substituents diminish the reactivity of the carbonyl group towards organic magnesium compounds.

Bromdiphenylpropiophenone, $(C_6H_5)_2CHCHBrCOC_6H_5$. — This substance was described in a previous paper. It is very easily obtained when the magnesium compound formed in the

reaction between benzalacetophenone and phenylmagnesium bromide is treated with bromine. The ketone and the magnesium compound were brought together in the usual way. After the mixture had stood for four hours an ethereal solution of bromine was added, drop by drop, until the color of bromine no longer disappeared. This required 2 atoms of bromine for each atom of magnesium used in the preparation of the magnesium compound. When the mixture was poured into dilute hydrochloric acid, the product separated in colorless needles. After one crystallization from a mixture of chloroform and alcohol it melted sharply at 173°. The amount of pure substance obtained was 98 per cent of that calculated from the amount of ketone that had been used.

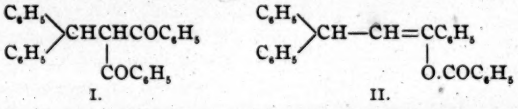
Benzoyltriphenylpropenol, $\begin{matrix} C_6H_5 \\ C_6H_5 \end{matrix}\!\!>\!CHCH\!=\!C\!<\!\begin{matrix} C_6H_5 \\ OCOC_6H_5 \end{matrix}$. — The magnesium compound that is formed in the reaction between benzalacetophenone and phenylmagnesium bromide reacts with benzoyl chloride in the cold. An ethereal solution of the chloride was added to a mixture of the ketone and phenylmagnesium bromide, that had been prepared in the usual way and then cooled to —20° in a freezing-mixture. Each drop of the chloride produced a white precipitate until the mixture contained 1 molecule of benzoyl chloride for each atom of magnesium. The solid cake obtained in this way was immediately treated with iced hydrochloric acid, washed thoroughly with cold hydrochloric acid, alcohol, and ether, and dried in a current of air. The crude product melted at 218° to 220°. It was dissolved in boiling chloroform and precipitated with hot absolute alcohol. It was thus obtained in long transparent needles that melted at 220°. An analysis gave the following results:

0.1901 gram substance gave 0.6005 gram CO_2 and 0.0962 gram H_2O.

	Calculated for $C_{33}H_{24}O_2$.	Found.
C	86.15	86.14
H	5.64	5.67

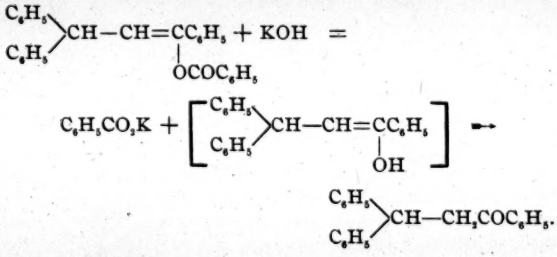
The substance is moderately soluble in boiling chloroform and in boiling benzene, very slightly soluble in alcohol, ether, and acetone.

The two possible formulas for a substance of this composition and origin are the following :



I. II.

Formula I. represents an alkyldibenzoylmethane—a β-diketone—which should be soluble in alkalies and which should form metallic derivatives. Formula II. represents an ester of benzoic acid which should be insoluble in alkalies, should form no metallic derivatives, but should be easily hydrolyzed to benzoic acid and diphenylpropiophenone :



The substance under consideration is insoluble in alkalies, even in a cold solution of sodium alcoholate in alcohol ; its solution in ether gives no copper compound when treated with copper acetate and ammonia ; it gives no sodium derivative when its solution in ether or benzene is heated with metallic sodium. When it is warmed with a strong solution of potassium hydroxide to which a few drops of alcohol have been added, it first turns intensely yellow, then melts down to a colorless oil that solidifies on cooling. The solution contains potassium benzoate. The insoluble substance is pure

diphenylpropiophenone. The structure of the substance is, therefore, represented by Formula II.

2. Benzalacetophenone and Methylmagnesium Iodide: Phenylbutyrophenone, $\begin{matrix} C_6H_5 \\ CH_3 \end{matrix}\!\!>\!CHCH_2COC_6H_5$.—The reaction was carried out in the usual way. The product crystallizes from alcohol in large, colorless plates melting at 74°. It is very soluble in alcohol and in ether, moderately soluble in ligroin.

0.1890 gram substance gave 0.5720 gram CO_2 and 0.1209 gram H_2O.

	Calculated for $C_{16}H_{16}O$.	Found.
C	85.71	86.00
H	7.14	7.10

The same substance has recently been described by Harries,[1] who obtained it by reducing dypnone:

$$\begin{matrix} C_6H_5 \\ CH_3 \end{matrix}\!\!>\!C{=}CHCOC_6H_5 + H_2 = \begin{matrix} C_6H_5 \\ CH_3 \end{matrix}\!\!>\!C.CH_2COC_6H_5.$$

Harries gives a little lower melting-point (72°), but the melting-point of the hydrazone made from my product was found to be 79°, as given by Harries.

The *oxime* was obtained by boiling an alcoholic solution of the ketone with hydroxylamine hydrochloride and potassium hydroxide. It is very soluble in alcohol and ether. It crystallizes from aqueous alcohol in needles that melt at 93°.

0.1543 gram substance gave 0.4550 gram CO_2 and 0.0992 gram H_2O.

	Calculated for $C_{16}H_{17}ON$.	Found.
C	80.33	80.42
H	7.11	7.14

II. Reactions with Benzalpropiophenone.

1. Preparation of Benzalpropiophenone.—A mixture of equivalent quantities of benzaldehyde and propiophenone was saturated with gaseous hydrochloric acid and allowed to remain

[1] Ann. Chem. (Liebig), 330, 223.

in ice-water for two days. The resulting greenish-yellow liquid was dissolved in ether, the ethereal solution washed with water, and extracted with a saturated solution of sodium bisulphite. After drying the solution with calcium chloride and removing the ether by distillation, there remained a pale-yellow oil that solidified almost completely when cooled in a freezing-mixture. The solid was recrystallized from low-boiling ligroin until it melted sharply at 83°. On analysis it was found to be benzylchlorpropiophenone,

$$C_6H_5CHClCHCOC_6H_5.$$
$$\quad\quad\quad

	Calculated for $C_{28}H_{30}N_2$.	Found. I.	Found. II.
C	84.61	84.29	84.50
N	6.42	6.80	6.64

2. Reaction between Benzalpropiophenone and Phenylmagnesium Bromide: Diphenylisobutyrophenone, $(C_6H_5)_2CH-CH(CH_3)COC_6H_5$.—The substances react energetically even at low temperatures. The ketone separates in crystalline form when the magnesium compound is decomposed with iced hydrochloric acid. The yield of pure ketone is 96 per cent of the calculated amount. An analysis gave the following results:

0.2325 gram substance gave 0.7522 gram CO_2 and 0.1403 gram H_2O.

	Calculated for $C_{22}H_{20}O$.	Found.
C	88.00	88.23
H	6.67	6.70

The ketone is readily soluble in alcohol and ether, moderately soluble in ligroin. It crystallizes from alcohol in needles, from ligroin in large transparent plates. It melts at 105°. It is not oxidized by cold permanganate.

The *oxime* was made by boiling an alcoholic solution of the ketone with hydroxylamine hydrochloride and potassium hydroxide. The product was precipitated with water and recrystallized from weak alcohol. It is readily soluble in alcohol and ether. It crystallizes in needles that melt at 145°.

0.1510 gram substance gave 0.4629 gram CO_2 and 0.0910 gram H_2O.

	Calculated for $C_{22}H_{21}ON$.	Found.
C	83.81	83.60
H	6.66	6.70

III. Reaction with Dypnone.

1. *Preparation.*—At first dypnone was prepared by the method of Delacre, but the yield was small—about 27 per

cent of the calculated amount. In a second preparation the method was modified as follows: One hundred grams of acetophenone were dissolved in an equal weight of glacial acetic acid and the solution saturated with hydrochloric acid at the temperature of running water. After standing for twenty-four hours the mixture was heated under diminished pressure in a water-bath for two hours and then fractionally distilled under a pressure of 28 mm. The yield was 62 grams of pure ketone. No acetophenone was left over. The residue, after distillation, was mainly triphenylbenzene.

2. Reaction with Phenylmagnesium Bromide: β,β-Diphenylbutyrophenone, $C_6H_5-\overset{\overset{C_6H_5}{\diagdown}}{\underset{\underset{CH_3}{\diagup}}{C}}-CH_2COC_6H_5$. — Phenylmagnesium bromide reacts much more slowly with dypnone than with the ketones previously described. The product crystallizes in thick prisms or tables melting at 103°. It is readily soluble in alcohol, acetone, and ether; moderately soluble in ligroin. It is not oxidized by cold permanganate.

0.1065 gram substance gave 0.3440 gram CO_2 and 0.0631 gram H_2O.

	Calculated for $C_{22}H_{20}O$.	Found.
C	88.00	88.08
H	6.66	6.58

The *oxime* was made by boiling an alcoholic solution of the ketone with hydroxylamine hydrochloride and potassium hydroxide. It is moderately soluble in alcohol and in ether. It crystallizes in needles melting at 163°.

0.1385 gram substance gave 0.4251 gram CO_2 and 0.0840 gram H_2O.

	Calculated for $C_{22}H_{21}ON$.	Found.
C	83.81	83.70
H	6.66	6.73

IV. Reaction with Benzalacetone.

Grignard allowed methylmagnesium iodide to react with

benzalacetone and obtained a hydrocarbon, presumably formed by loss of water from an intermediate tertiary alcohol. When phenylmagnesium bromide reacts with benzalacetone the changes observed are exactly like those observed in the case of benzalacetophenone. A colored magnesium compound separates out and redissolves when the liquid is shaken. After the mixture has stood for some time a new magnesium compound separates as a colorless paste. No solid substances are obtained when this paste is decomposed with ice and hydrochloric acid. The product is an oil from which no solid substances could be obtained, either by prolonged exposure to low temperatures or by distillation with steam. When heated under diminished pressure the liquid remained clear until the temperature of the bath was 178°. At this point it suddenly became cloudy while water began to condense in the receiver. This indicated that the substance was not a ketone but an alcohol, and a test with permanganate showed that, unlike the ketones described above, it reduced a cold neutral solution of potassium permanganate. A new preparation of the substance was therefore made and oxidized as follows:

Twenty grams of the substance were suspended in a liter of water that was kept at 0° with cracked ice. To this liquid a cold saturated solution of potassium permanganate was added, in small portions, until the color remained after vigorous shaking. The oxides of manganese were filtered off and the filtrate acidified. The precipitate that formed was pure benzoic acid. The filtrate from the benzoic acid was evaporated to 250 cc. and cooled. The crystals that separated were again almost pure benzoic acid. The filtrate from this was distilled in a current of steam to remove the last traces of benzoic acid, and, finally, evaporated to a very small volume. The acid that separated on cooling was almost pure atrolactic acid. After one recrystallization from water it melted at 89° to 90°; after driving off the water of crystallization on the water-bath it melted at 95°. When boiled with concentrated hydrochloric acid and distilled with steam atropic acid, melting at 104°, passed into the distillate.

These products of oxidation—benzoic acid and atrolactic

acid—show that the substance obtained from benzalacetone is the tertiary alcohol, $C_6H_5CH:CHC(C_6H_5)(CH_3)OH$, the oxidation taking place according to the equation:

$$C_6H_5CH:CHC(C_6H_5)(CH_3)OH + 4O = C_6H_5CO_2H + CO_2H.C(C_6H_5)(CH_3)OH.$$

V. Reaction with Cinnamic Aldehyde and α-Bromcinnamic Aldehyde.

Sand and Singer treated cinnamic aldehyde with methylmagnesium iodide and obtained a secondary alcohol. The main object of the following experiment was to ascertain whether the reaction goes exclusively in this direction. Cinnamic aldehyde was added to an ethereal solution of phenylmagnesium bromide that was kept at —22°. The product, isolated in the usual way, was an oil that did not solidify in a freezing-mixture. One-half of the entire product was oxidized with potassium permanganate, the remainder boiled with hydroxylamine hydrochloride and potassium hydroxide. The oxidation was carried out in benzene solution. The substance was dissolved in twenty times its volume of pure benzene and this solution shaken with an iced 1 per cent solution of potassium permanganate that contained 1 atom of available oxygen for every molecule of the substance. After complete reduction of the permanganate, the benzene solution was separated and the benzene removed by distillation from the water-bath. The residue was a yellow oil that partially solidified on standing. The yellow solid was filtered off and recrystallized from ligroin. After one crystallization it melted at 50°, the melting-point of benzalacetophenone. The oil was distilled with steam. Only brombenzene passed into the dis-

tillate. The residue solidified completely on cooling. It was almost pure benzalacetophenone. The aqueous solution left after separating the benzene contained only benzoic acid. Judging by the behavior on oxidation, the product contained only the secondary alcohol, $C_6H_5CH : CHCHOHC_6H_5$, which was oxidized as follows:

$$C_6H_5CH : CHCHOHC_6H_5 + O = C_6H_5CH : CHCOC_6H_5 + H_2O.$$

No oxime was obtained in the experiment with hydroxylamine.

The carbonyl group of α-bromcinnamic aldehyde is much less reactive than that of cinnamic aldehyde: it does not condense with acetophenone in the presence of alkalies, nor with malonic acid in the presence of acetic acid or acetic anhydride. It seemed possible, therefore, that the aldehyde might take up organic magnesium compounds in the 1,4-positions. The experiment was carried out exactly like that with cinnamic aldehyde and the result was the same. No trace of a saturated aldehyde could be detected in the product.

CHEMICAL LABORATORY,
BRYN MAWR COLLEGE.

Preliminary Report from the Kent Chemical Laboratory of the University of Chicago.

THE CONSTITUTION OF PURPURIC ACID AND OF MUREXIDE.[1]

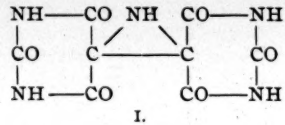
BY MAX SLIMMER AND JULIUS STIEGLITZ.

Of the many remarkable products that may be obtained from uric acid, perhaps none more excited the interest of older chemists, or has proven more valuable as the basis of a sensitive qualitative test in the physiological and chemical study of this group, than murexide. First observed by Scheele, Prout[2] later isolated it in crystalline form by the oxidation of uric acid with nitric acid and subsequent treatment with ammonia. To the crystalline substance so obtained he gave the name of purpurate of ammonia, and by

[1] All the experimental work described in this paper was carried out by Dr. Slimmer. J. STIEGLITZ.

[2] Phil. Trans., 1818, p. 420.

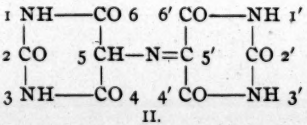
double decomposition he formed a number of other salts. By treatment with acids he obtained from the highly colored salts a colorless substance which he considered to be the free purpuric acid. Liebig and Wöhler[1] soon showed that this body was not the only product of the action of acids on the purpurates. Their investigations on the action of ammonia on alloxantin and on the oxidation of uramil to murexide include almost all that we know to this day of the chemistry of these substances. It was, however, left for Beilstein[2] to show that the reaction between alloxan and uramil is not as Liebig and Wöhler assumed, an oxidation, but a true condensation, occurring in alkaline solutions. Free purpuric acid is not stable, but on liberation by acids in aqueous solutions is at once decomposed into uramil and alloxan. As in best accord with these facts, Beilstein proposed the following constitution,



I.

for purpuric acid, which is now commonly accepted.[3]

In his lectures one of us has already for many years expressed great doubt as to the correctness of this constitution for purpuric acid and has suggested the following linking of the carbon and nitrogen atoms as more clearly expressing its reactions:[4]



II.

Its formation from uramil and alloxan in alkaline solutions

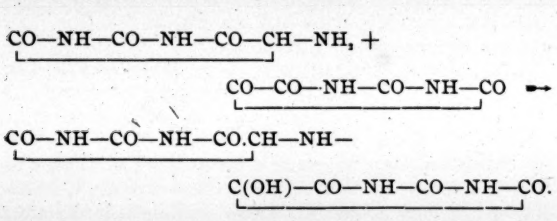
[1] Ann. Chem. (Liebig), **36**, 241 (1838).

[2] *Ibid.*, **107**, 241 (1858).

[3] Compare Beilstein, Vol. I., 1340. Also Meyer and Jacobson, Vol. I., 1082. See, however, Bernthsen's "Lehrbuch der organischen Chemie," p. 291.

[4] See page 665 in regard to the question of pseudopurpuric acid and the constitution of the metal salts of the acid.

appeared as the result of a condensation of an amine, uramil, with a ketone, alloxan, to form a "ketone ammonia" which by subsequent loss of water produces the imide (II.):

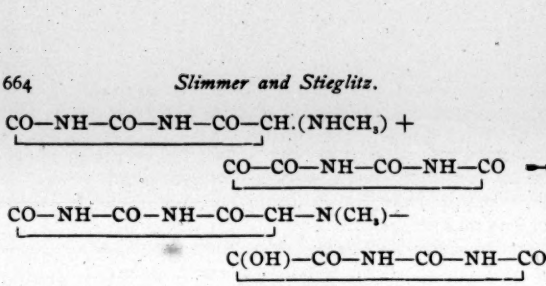


III.[1]

Especially the most striking property of purpuric acid, its great instability towards aqueous acids, is well expressed by the above formula. Imides, such as the imidoethers and imidoquinones, are well known to be readily saponified by aqueous acids. As purpuric acid is itself a fairly strong acid, its instability in the free state in the presence of water follows as a matter of course.[2] As will be shown presently, we have been able to prove conclusively that Beilstein's constitution (I.) for purpuric acid is wrong, and that murexide as dried at 100° is really the ammonium salt of an acid whose constitution is expressed in (II.). The interesting discovery was, moreover, made that there is a marked tendency for the condensation between uramil and alloxan to stop at the intermediate carbonyl ammonia stage (III.) and that freshly prepared murexide contains 1 molecule of water, which it loses at 100°. That this molecule of water is not water of crystallization but water used to form the carbonyl ammonia group was shown by the fact that it was possible to prepare salts of intensely colored derivatives of purpuric acid from 7-alkyluramils and alloxan; for instance, in the presence of ammonia, salts were obtained by the following condensation:

[1] Bernthsen's "Lehrbuch" gives III. as a possible constitution for purpuric acid.

[2] Attempts will be made to isolate free purpuric acid in the absence of water and experiments made on the nature of the substances in aqueous solution formed immediately after the addition of an acid to a purpurate. J. Stieglitz.



IV.

These substances cannot lose the molecule of water in question, but otherwise they resemble the purpurates, notably as regards intensity of color and sensitiveness to acids. This tendency to form carbonyl-ammonia derivatives is in perfect accord with the general behavior shown by mesoxalic acid and mesoxalylurea (alloxan) in forming stable hydrates of the carbonyl group, $:C(OH)_2$. It is probably owing to this tendency, too, that purpuric acid shows such extreme sensitiveness to acids, for it is well known that carbonyl ammonias, like aldehyde ammonias, are even more rapidly decomposed by acids than imido compounds are hydrolyzed.

The proof that the true constitution of purpuric acid is represented by II.[1] (respectively III. for the hydrate) and not by I. is based on the following lines of experiment:

1. Constitution I. represents a symmetrical molecule; the same ammonium salt of 1,3-dimethylpurpuric acid ought to result on condensing either 1,3-dimethyluramil with alloxan or uramil with 1,3-dimethylalloxan, if Beilstein's constitution is correct. II. and III. are unsymmetrical and enable us to predict that the ammonium salts of two isomeric dimethylpurpuric acids (1,3 and 1′,3′) would result from the above condensations. Our experiments show that two isomers are actually obtained.

2. According to II. (and III.) the presence of the hydrogen attached to the carbon atom, 5, of the uramil group is not essential for the formation of condensation-products of the nature of purpuric acid, while according to Beilstein's formula it is. We have found that 5-amido-6-methyl uracil

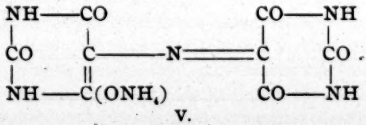
[1] II. and III. are "pseudo" purpuric acids as will be explained further on.

condenses with alloxan to form a deep-red compound of the general character and composition of the purpurates; the uracil has the amine group attached to a tertiary carbon atom holding no hydrogen.

3. The purpurate obtained from 7-methyluramil and alloxan in ammoniacal solution has the composition $C_9H_{13}O_7N_6$, even when dried at 100°, as required by constitution III. There is no tendency shown to lose water to form a triatomic ring as required by I., even when there is no opportunity to lose water to form an imide according to II.

We may therefore consider the evidence as conclusive that II. and not I. represents the constitution of purpuric acid and its anhydrous salts, as far as the linking of the carbon, nitrogen, and oxygen atoms is concerned, and that III. represents the constitution of the hydrated salts.

We have also begun an investigation of the interesting question as to which hydrogen atom in purpuric acid is involved in the formation of its monobasic salts and particularly as to the constitution of these salts. The evidence points to the conclusion that the more ionizable hydrogen is the one in position 5 of the uramil ring, and that in the salts the metal is bound, as we expected, to the oxygen of the neighboring carbonyl group (4 or 6). We have then for the constitution of murexide in its anhydrous form:



V.

Whether purpuric acid in the free state would exist in the pseudo form II. (respectively III.), as is likely, or as the true acid corresponding to V. is a question which can only be answered when the acid has been isolated. The proof that the metal is attached to oxygen in the purpurates rests on the fact that silver purpurate, when treated with methyl iodide, produces a methyl ether which with dry hydrogen chloride at 125° to 130° gives a large quantity of methyl chloride. As is

well known, this is a characteristic reaction of a methoxyl group, $—OCH_3$; under the conditions named, neither methylimide groups nor methylcarbin groups, $:C—(CH_3)$, would lose methyl chloride. That we are justified in concluding that the metal is bound to oxygen from the formation of an oxygen ether from the silver salt follows from the work of Nef[1] on the salts of malonic and acetoacetic esters, from various investigations on the silver salts of acylamides,[2] and from the work of Hantzsch[3] on pseudo-acids and their salts, work which by physico-chemical determinations fully established the conclusions based in the previous investigations on synthetic results. They all agree in showing that silver salts produce oxygen ethers when the salts hold the metals attached to oxygen.

Sodium and potassium salts, on the other hand, while having the same constitution as the silver salts, *i*, *e*., holding the metal attached to oxygen,[4] are known to give as a rule with methyl iodide methyl derivatives of the tautomeric or pseudoform of the acid,[5] and this fact is very valuable in determining whence the ionizable hydrogen of the true acid is obtained. Applying this method to the purpurates we have found that potassium purpurate, when treated with methyl iodide, produces a methyl derivative from which hydrogen chloride does not split off methyl chloride, and from which by destructive treatment with concentrated aqueous hydrochloric acid no methylamine could be obtained. It is almost certain, therefore, that the methyl group has gone into position 5 of the uramil half of purpuric acid and that, consequently, it is the hydrogen atom in this position of the pseudo-acid, which is used to form with either of the (identical) neighboring car-

[1] Ann. Chem. (Liebig), **266**, 66 (1891); **276**, 235 (1893).

[2] Tafel and Enoch: Ber. d. chem. Ges., **23**, 104 and 1550 (1890); Comstock and Kleeberg: This JOURNAL, **12**, 493 (1890); Comstock and Wheeler: *Ibid.*, **13**, 520 (1891); Stieglitz and Dains: J. Am. Chem. Soc., **21**, 187 (1899).

[3] Ber. d. chem. Ges., **32**, 575 (1899).

[4] This is proved conclusively by Hantzsch, *Loc. cit.*

[5] This is so in all of the cases quoted above. The reaction is analogous to the fact established by Wheeler that imidoethers, of the formula $RC(:NH)OCH_3$, which correspond to the salts, $RC(:NH)OK$, give with methyl iodide acylmethylamines, $RCO.NHCH_3$. It is probable that methyl derivatives of the pseudo-acids would result from the treatment with methyl iodide of all oxygen ethers of the corresponding true acids, the former being the more stable compounds.

bonyl groups the true acid group : C(OH). We shall endeavor to confirm this conclusion by determining whether alanine, $CH_3CH(NH_2)CO_2H$, is formed by decomposition of the product. The same conclusion as to the hydrogen atom of purpuric acid which is taken to form the metal salts (V.) follows indirectly from the fact that 1,3-dimethylalloxan and 1,3-dimethyluramil condense to form 1,3,1′,3′-tetramethylpurpurates (murexoin).

We suspect that the change of structure from the pseudo-form, II. or III., to that of the salts, V, may have an important connection with the question of color in this group. The following compounds which have a structure corresponding to V. are intensely colored : 1. The purpurates like murexide. 2. The oxygen ester of purpuric acid as obtained from the silver salt and methyl iodide. 3. The salts of 7-alkylpurpuric acids. 4. The condensation-product of 4-methyl-6-aminouracil and alloxan. On the other hand, the product obtained by methylation of the potassium salt of purpuric acid is pale-yellow, and, as we have just seen, it is most likely-5-methylpurpuric acid, a derivative of the pseudo-form, II. Alloxantin, which, as we shall presently show, almost surely has a constitution entirely analogous to the pseudo-form, III., is also colorless. It is possible, therefore, that purpuric acid itself will be found to be colorless, should it be more stable in the pseudo-form, II., than in the acid form, V. The residue left when methyl purpurate (the red oxygen ether) is treated with dry hydrogen chloride at 130° is white and gives instantly a deep-red solution on being treated with ammonia ; the white substance is most likely pseudopurpuric acid, II., itself, but further investigation will be necessary before we can speak authoritatively on this point or the question of color in this group, and we wish to reserve the field for further development in this laboratory.[1]

Another condensation-product of alloxan that is closely related to murexide is alloxantin. Formed by the condensation of alloxan with dialuric acid (tartronylurea), it can also be prepared by the reduction of alloxan by means of hydrogen sulphide. Unlike purpuric acid, it is stable only in acid

[1] Mr. R. W. Noble is continuing the investigation under the direction of one of us.

solutions, alkalies decomposing it at once into salts of dialuric acid and alloxan. Its seemingly close relation to purpuric acid is shown by its easy conversion into murexide through the action of ammonia. This and its formation by the reduction of alloxan has been expressed by the following constitution which has commonly been accepted :

$$\underline{CO—NH—CO—NH—CO—C(OH)—}$$
$$\underline{C(OH)—CO—NH—CO—NH—CO}. \quad \text{VI.}$$

Its sensitiveness to alkalies and its relation to purpuric acid suggest the following constitution :

$$\underline{CO—NH—CO—NH—CO—CH—O—}$$
$$\underline{C(OH)—CO—NH—CO—NH—CO}. \quad \text{VII.}$$

According to this view it would appear as the hemi-acetal of the alcohol (or phenol), tartronylurea, and the ketone, mesoxalylurea. Such an acetal would readily form with ammonia uramil and alloxan, which would then condense in the presence of an excess of ammonia to murexide. With bases it would give dialurates and salts of alloxan. The existence of tetramethylalloxantin (amilinic acid) and its conversion into tetramethylmurexide through the action of ammonia reduces the question of the constitution of alloxantin to the determination of the function of the hydrogen atoms in the tartronyl radical. The constitution of purpuric acid having been determined, the investigation will be extended to bring more rigorous proof of the correctness of the constitution VII., suggested for alloxantin.

EXPERIMENTAL PART.

The Asymmetry of the Purpuric Acid Molecule.

Ammonium 1,3-Dimethylpurpurate,

$$\underline{CO.NCH_3.CO.NCH_3.C(ONH_4):C}—N:\underline{C.CO.NH.CO.NH.CO},$$

and Ammonium 1',3'-Dimethylpurpurate,

$$\underline{CO.NH.CONH.C(ONH_4):C}—N:\underline{C.CO.NCH_3.CO.NCH_3.CO}.$$

—Eight grams of 1,3-dimethyluramil, prepared from caffeine

according to Fisher's modification of Techow's method,[1] were added to 40 cc. of boiled water at the temperature of 70°, a stream of hydrogen being run through the flask to replace the air. Sufficient ammonium carbonate was now added to dissolve the uramil and the mixture added to 7 grams (1 molecule = 6.65 grams) of alloxan dissolved in 5 cc. of hot water. After the mixture was allowed to stand in the cold for half an hour, the crystals were filtered off and recrystallized from hot water. Yield, 9 grams.

For analysis the substance was dried at 100°.

0.1938 gram substance gave 0.0517 gram N.

0.2348 gram substance gave 0.3297 gram CO_2 and 0.0795 gram H_2O.

	Calculated for $C_{10}H_{12}O_8N_8$.	Found.
C	38.46	38.30
H	3.85	3.79
N	26.92	26.71

The substance crystallized in small, four-sided prisms, very similar to the ordinary murexide only a little yellower in luster.

Eight and five-tenths grams of the above compound (1,3-dimethylpurpurate of ammonium) were suspended in 30 cc. of water and the air in the flask replaced by hydrogen. The mixture was now made slightly acid with hydrochloric acid and warmed to 70° until it was of a pale-yellow color. After the mixture was cooled to 0°, the precipitate was filtered off, washed with a little cold water, and dried in a vacuum over sulphuric acid. Yield, 2.41 grams. Analysis showed that the body is dimethyluramil:[2]

0.4880 gram substance gave 0.1180 gram N.

[1] Ber. d. chem. Ges., 28, 2475 (1895).

[2] In order to avoid repetition we wish to remark here that the bodies described in this paper, almost without exception, have no sharp melting-point but decompose at higher temperatures without melting. The investigation was for this reason rendered more difficult as the bodies obtained in a reaction as well as the recovered starting material could only be identified by analysis. Carbon and hydrogen were determined according to Dennstedt (Ztschr. anal. Chem., 41, 525 (1902)). The nitrogen was determined according to Kjeldahl or by distillation with soda-lime, the strength of the acid used having been determined in terms of milligrams of nitrogen.

	Calculated for $C_4H_9O_2N_3$.	Found.
N	24.56	24.19

The filtrate and washings were now made more strongly acid and treated with hydrogen sulphide. There were obtained in this way 3.7 grams of alloxantin. After drying over sulphuric acid it gave the following analysis:

0.3172 gram substance gave 0.0557 gram N.

	Calculated for $C_8H_4O_8N_4 + 2H_2O$.	Found.
N	17.39	17.53

Treated with hydrochloric acid (sp. gr. 1.19) for five hours at 160°, it was completely decomposed, and after evaporation of the excess of acid and distillation with caustic potash, the distillate gave no test for methylamine.

In the same way uramil was condensed with dimethylalloxan to give another purpurate of ammonia (1′,3′-dimethylpurpurate of ammonia). This was so similar to its isomer in solubility and general appearance that had it not given other products on treatment with acids, it might have been taken to be identical with it.

Seven grams decomposed by the action of dilute hydrochloric acid gave a uramil which, on account of its difficult solubility, was at once recognized as ordinary dialuric acid amide. To further characterize the same it was also decomposed with strong hydrochloric acid as described above and tested for methylamine. With chloroform and caustic potash it did not give the faintest odor of methyl isocyanide.

From the mother-liquid there was thrown out by the action of hydrogen sulphide a white crystalline solid which on recrystallization from hot water proved, on analysis, to be amilinic acid (tetramethylalloxantin). For analysis the same was dried at 100°.

0.2183 gram substance gave 0.3354 gram CO_2 and 0.0783 gram H_2O.

0.2990 gram substance gave 0.0477 gram N.

	Calculated for $C_{12}H_{11}O_8N_4$.	Found.
C	42.10	41.90
H	4.09	4.01
N	16.37	15.96

The above experiments show conclusively that the molecule of purpuric acid is not symmetrical, for the two acids obtained above are different, as shown by their having different decomposition-products.

The Condensation of 5-Amido-4-methyluracil with Alloxan.

4-Methyluracilimidoalloxan,

$CO.NH.CONHC(CH_3) : C—N=C.CONHCONHCO.$—Four

grams of the uracil[1] were placed in a flask with 4 grams of alloxan, and, after the addition of 25 cc. of water, were heated on the water-bath for twenty minutes. The mixture soon became a deep wine-red color and after the addition of 50 cc. of strong alcohol and cooling deposited a heavy, dark-red precipitate. This product could not be obtained in well-defined crystals; the analysis proves that the product has the composition $C_9H_7O_8N_5$. For analysis it was dried at 100°.

0.2359 gram substance gave 0.3453 gram CO_2 and 0.0544 gram H_2O.

0.2866 gram substance gave 0.0769 gram N.

	Calculated for $C_9H_7O_8N_5$.	Found.
C	40.07	39.92
H	2.64	2.58
N	26.41	26.84

In amidomethyluracil the amido group is attached to a tertiary carbon atom. That it forms a murexide-like condensation-product with alloxan is good evidence of the incorrectness of Beilstein's formula.

The Action of Ammonia and Primary Amines on Alloxantin and the Significance of the Water of Crystallization in the Salts of Purpuric Acid.

Gmelin[2] first prepared murexide by heating alloxantin in a

[1] Behrend: Ann. Chem. (Liebig), 231, 250 (1885).

[2] Gmelin, V., 326.

stream of dry ammonia gas at 100° and recommends this as the best method of preparing the same. Later Rochleder[1] prepared murexoin (tetramethylpurpurate of ammonia) in the same way. We used the method a number of times for preparing murexide and found that it gave very good yields if absolute purity was not desired. In order to remove the last traces of alloxantin frequent recrystallization is, however, necessary, and much loss of material results. The following improvement of Gmelin's method which gives quantitative yields of almost pure material can be highly recommended:

Twenty grams of finely powdered and dried alloxantin are placed in a large tube and after the addition of 2 gram-molecules of ammonia dissolved in 100 cc. of absolute alcohol, the tube is sealed and heated to 100° for ten hours, being shaken from time to time. At the end of this time the alloxantin is completely converted into anhydrous murexide. It need only be filtered from the mother-liquid and washed with a little alcohol and it is then perfectly pure. Yield, about 20 grams.

When examined in the light of the formula that we have proposed for alloxantin the mechanism of this reaction is very simple. Alloxantin, the dialuric acid ester of alloxan, gives with ammonia the acid amide, uramil, and alloxan; these then condense in the presence of a second molecule of ammonia, giving purpurate of ammonia. This tendency of alloxantin to react with amines has long been known but a plausible explanation has never been offered before. For instance, boiled with ammonium chloride it gives uramil, alloxan, and hydrochloric acid;[2] in the same way, boiling it with cyanamide converts it into pseudouric acid and alloxan.[3] In a later paper this very fruitful reaction will be more carefully treated and some of the simpler esters of dialuric acid as well as the synthesis of dialuric acid amides (uramils) and ureids (pseudouric acids) more fully described.

Beilstein and the older chemists who have analyzed the salts of purpuric acid, dried the same at 100°, and found that they were anhydrous with the exception of the silver salt

[1] Jsb., 1850, p. 436. See also Brunn: Ber. d. chem. Ges., 21, 514 (1888).
[2] Mulder: Ber. d. chem. Ges., 6, 1235 (1873).
[3] Mulder: *Ibid.*, 6, 1236 (1873).

which has 0.5 molecule of water. Matignon[1] first described murexide as containing 1 molecule of water, which it loses already below 100°. We have again analyzed a number of purpurates and find that they all, when freshly prepared, contain 1 molecule of water, which they lose at ordinary temperature on standing.

Ammonium purpurate hydrate, prepared as described above and recrystallized from water containing a few drops of ammonia, was spread on a clay plate for twenty minutes at room temperature and analyzed:

0.2711 gram substance gave 0.3164 gram CO_2 and 0.0786 gram H_2O.

0.4247 gram substance gave 0.1195 gram N.

	Calculated for $C_8H_{10}O_7N_6$.	Found.
C	31.78	31.83
H	3.31	3.24
N	27.81	28.14

Potassium purpurate hydrate, prepared as described below, was dried in the same way and analyzed as follows:

0.5873 gram substance gave 0.1653 gram K_2SO_4.

	Calculated for $C_8H_6O_7N_5.K$.	Found.
K	12.38	12.64

The so-called water of crystallization present in the salts of purpuric acid is constitutional (see the introduction) and has the same significance as has the water in chloralhydroxylamine, which is also lost at ordinary temperature when that body goes over into chloraloxime. The group of purpuric acids that can be prepared from primary amines and alloxantin, that is, the condensation-products of alloxan and 7-alkyluramils, cannot lose water and have no water that could be mistaken for water of crystallization. Of this group the 7-methyl and the 7-ethylpurpurates of ammonia were prepared.

[1] Ann. chim. phys. [6], 28, 346 (1893). He gives no analytical data in support of his views.

Ammonium 7-Methylpurpurate Hydrate,

$CO.NH.CO.NH.C(ONH_4) : C—$

$(NCH_3).C(OH)CONHCONH.CO.—$

This body was prepared in a number of ways:

1. By the condensation of 7-methyluramil and alloxan.

7-Methyluramil was prepared by the action of methylamine sulphite on alloxan as recommended by Fischer.[1] We worked at a much lower temperature than Fischer did and used pure methylamine, but even then did not get a perfectly pure product, as was shown by its analysis and by its reaction with potassium cyanate, which gave some pseudouric acid. The formation of a slight amount of ordinary uramil is easily explained on the assumption that the methylamine decomposed some of the alloxan with the formation of ammonia. For analysis it was dried at 100°.

0.2785 gram substance gave 0.3864 gram CO_2 and 0.1023 gram H_2O.

0.4731 gram substance gave 0.1282 gram N.

	Calculated for $C_5H_7O_3N_3$.	Found.
C	38.21	37.84
H	4.45	4.10
N	26.75	27.12

Absolutely pure *7-methyluramil* can be obtained by the action of methylamine at low temperatures on alloxantin or other esters of dialuric acid. Some prepared in this way was analyzed as follows:

0.2034 gram substance gave 0.2839 gram CO_2 and 0.0794 gram H_2O.

0.3390 gram substance gave 0.0905 gram N.

	Calculated for $C_5H_7O_3N_3$.	Found.
C	38.21	38.07
H	4.45	4.36
N	26.75	26.62

Four grams of 7-methyluramil were dissolved in a little

[1] Ber. d. chem. Ges., 30, 561 (1897).

more than 1 molecule of aqueous ammonia (30 cc.) and the solution added to a solution of 3.6 grams of alloxan in 20 cc. of water. After the mixture was warmed for five minutes on the water-bath, some ammonium carbonate was added to aid crystallization and the mixture allowed to cool. Yield 3.1 grams. The body crystallized in small, four-sided prisms, which resembled the ordinary murexide so closely that they could only be distinguished by analysis. For analysis the body was dried at 100°:

0.2103 gram substance gave 0.2609 gram CO_2 and 0.0719 gram H_2O.

0.4181 gram substance gave 0.1105 gram N.

	Calculated for $C_8H_{12}O_7N_6$.	Found.
C	34.17	33.83
H	3.80	3.84
N	26.58	26.41

2. By the oxidation of 7-methyluramil with mercuric oxide.

Five grams of methyluramil and 4.5 grams of mercuric oxide were suspended in 100 cc. of water and the mixture heated to boiling. As the filtrate could not be made to crystallize on account of the great solubility of the methylammonium salt of methylpurpuric acid, 15 grams of ammonium carbonate were added. A heavy precipitate of the ammonium salt was formed. Yield, 2.3 grams.

0.3329 gram substance gave 0.0876 gram N.

	Calculated for $C_8H_{12}O_7N_6$.	Found.
N	26.58	26.32

3. The action of methylamine on alloxantin.

Fifteen grams of powdered and dried alloxantin were sealed into a glass tube with 100 cc. of absolute alcohol and 2 molecules of methylamine. After standing at room temperature for twenty-four hours the mixture was heated for five hours to 100°. Fourteen and eight-tenths grams of a dark-red solid were obtained, but on account of the great solubility of this methylammonium salt and because some decomposition had taken place, it could not be obtained sufficiently pure for

analysis. The body was therefore converted into the ammonium salt as follows: Ten grams of the solid were dissolved in 50 cc. of hot water and 1.5 grams of alloxan added. After the addition of 15 grams of ammonium carbonate the ammonium salt fell out as a mass of brilliant, greenish crystals. Yield, 7.3 grams.

Ammonium 7-Ethylpurpurate Hydrate,

$$\underset{\llcorner\!\!\lrcorner}{CONHCONH.C(ONH_4)}:C.\underset{\llcorner\!\!\lrcorner}{NC_2H_5.C(OH)}.CONHCONHCO.$$

—7-Ethylpurpurate of ammonia was prepared from alloxantin and ethylamine in the same way as the above methyl compound. It was found, however, that the ethylamine acted on the alloxan formed, even more than did the methylamine. On this account it was found not advisable to use more than 1 molecule of ethylamine in the reaction. In this way the alloxantin was converted almost quantitatively into 7-ethyluramil and alloxan. Ten grams of alloxantin were suspended in 100 cc. of absolute alcohol and after the addition of 1 molecule of ethylamine, heated for seven hours to 100°. On opening the tube the solid contents were found to weigh 6.7 grams and in the alcohol were found 4.2 grams of alloxan. The 7-ethyluramil was at once converted into the corresponding purpurate of ammonia as follows: It was dissolved in 80 cc. of hot water and after the same had cooled to 70°, 5 grams of alloxan and 10 grams of powdered ammonium carbonate were added. On cooling, the ethylpurpurate of ammonia crystallized out. Yield, 6.23 grams. For analysis it was dried at 100°.

0.1978 gram substance gave 0.2621 gram CO_2 and 0.0760 gram H_2O.

0.3113 gram substance gave 0.0810 gram N.

	Calculated for $C_{10}H_{14}O_7N_6$.	Found.
C	36.36	36.15
H	4.24	4.30
N	25.45	26.01

The body resembles the other purpurates so closely that only the analysis and the decomposition-products can be used

as a means of identification. Treated with acids, it gives 7-ethyluramil. This body, which is even more soluble than the methyl body, will be described at greater length in a later paper.

The Position of the Metal in the Salts of Purpuric Acid.

Purpuric acid forms only one series of well-defined salts, acting in this respect like a monobasic acid. The potassium and silver salts were methylated in order to get some idea as to the position of the metal in them.

The potassium salt of purpuric acid has been prepared by boiling murexide with a large excess of potassium chloride. This method does not give a very pure product unless the same is frequently recrystallized with great loss of material. We can recommend the following method of condensing uramil and alloxan in the presence of caustic potash as giving good yields of very pure salt.

The uramil used in this and the other experiments was made from malonic ester. The same was converted into barbituric acid by a modification of Michael's method.[1] We believe that the method as described by Michael will not give over 40 per cent to 50 per cent yields. In the same way Tafel and Weinschenk[2] were unable to obtain more than 40 per cent by their modification of the same method. By working at slightly higher temperatures and under pressure we found that one obtains almost a quantitative yield.

Four and six-tenths grams of metallic sodium are dissolved in 100 cc. of absolute alcohol and to the hot solution 12 grams of powdered urea are quickly added. In the mean time one has prepared a solution of 32 grams of malonic ester in 50 cc. of alcohol and, as soon as the urea is all dissolved, the two solutions are rapidly mixed and poured into a large tube or pressure-bottle and sealed up. The mixture becomes thick at once, and if it is heated to 100° for seven hours the reaction is found to be complete. The tube is now opened and the con-

[1] J. prakt. Chem., **38**, 456.
[2] Ber. d. chem. Ges., **33**, 3383 (1890).

tents are dissolved in the least amount of hot water and decomposed with acids.[1] Yield, 94 to 97 per cent.

The barbituric acid was converted into potassium violurate by means of potassium nitrite and acetic acid and the violurate reduced to uramil by hydriodic acid and phosphonium iodide. We obtained in this way 70 per cent of the malonic ester converted into uramil. To convert the same into potassium purpurate it was dissolved in one gram-molecule of caustic potash in fifty times its weight of water, the air being replaced by a stream of hydrogen. When it had all dissolved, which sometimes required heating, the warm solution was poured into a solution of alloxan in ten times its weight of water. The solution of alloxan must not be poured into the alkaline solution of uramil as the free alkali then decomposes the purpurate formed.

5-Methylpurpuric Acid,

$$\underline{CO.NH.CO.NH.CO.C(CH_3).N} : \underline{C.CO.NH.CO.NH.CO.} —$$

The potassium salt was dried at 100° and methylated by being heated to 100° for five hours with one gram-molecule of methyl iodide diluted with an equal volume of absolute ether. The tube was now opened and the solid filtered off and a portion recrystallized from water and analyzed :[2]

0.2121 gram substance gave 0.2965 gram CO_2 and 0.0485 gram H_2O.

0.3172 gram substance gave 0.0802 gram N.

	Calculated for $C_5H_7O_4N_3$.	Found.
C	38.43	38.12
H	2.49	2.57
N	24.91	25.27

[1] Monomethylbarbituric acid was prepared in the same way. As starting material we used the mixture obtained on methylating malonic ester with methyl iodide according to Conrad. This contains only about 80 per cent of monomethylmalonic ester together with unchanged ester and some dimethyl ester. The yield of pure material after recrystallizing from water was 17.2 grams out of 35 grams of mixed esters or calculated for the monomethyl ester, 80 per cent of the theory. It possessed the crystal form and other properties ascribed to it by Franchimont and Klobbie, but melted at 202° (corr.), 10° higher than found by these investigators.

[2] The methyl ether as prepared was pale-yellow, but on recrystallizing became red. This may have been due to some decomposition, and as it is of importance in view of the theory that we have proposed for the color of these bodies, we shall try to prepare 5-methyluramil and condense it with alloxan.

O-4-Methyl purpurate,

$$\underline{CO.NHCONHC(OCH_3)} : C.N : \underline{C.CO.NH.CO.NH.CO.} -$$

Silver purpurate, prepared by the precipitation of a solution of murexide with silver nitrate, was dried at 100° and then heated to 100 for three hours with 1 gram-molecule of methyl iodide and the same volume of absolute ether. The tube was now opened and the mixture of silver iodide and the methyl ether filtered off. Attempts to purify the ether by recrystallization from water or alcohol failed on account of the great instability of the body. It is slowly hydrolyzed even by boiling water.

0.174 gram of silver purpurate that had been dried at 100° were methylated as above and the product carefully collected and placed in a small flask which was connected with an azotometer filled with strong caustic potash. After the air had been expelled from the apparatus by means of carbon dioxide, dry hydrogen chloride was passed through, the flask being heated in an oil-bath. At 125° to 130° a gas, methyl chloride, was given off which was not absorbed by caustic potash and which burned, on being ignited, with a lambent green flame. The theory demanded 14 cc. of gas; there were obtained 6.8 cc.

In view of the fact that methyl chloride is quite soluble in water, the above experiment would tend to show that when the silver salt of purpuric acid is methylated the oxygen ether is formed. The body is colored very deep red but on being dissolved in hot water as described above, it is rapidly hydrolyzed and forms a colorless solution.

The work on the salts of purpuric acids will be continued. Special stress is to be laid on the cause of color in this group and on the question of the existence of free purpuric acid.

CHICAGO, March 10, 1903.

NOTE.

To the Editor:

In the March[1] issue of your JOURNAL was published an article about "The Action of Ammonia upon Copper Sulphate Solutions," by James Locke and the undersigned. I have worked out the experimental part of the investigation, but I wish to state that I am not responsible for the theoretical speculations advanced in the article, this not being referred to me before printing.

Very respectfully,

JACOB FORSSELL.

REVIEWS.

DIE WISSENSCHAFT. Sammlung naturwissenschaftlicher und mathematischer Monographien. Heft 1.

UNTERSUCHUNGEN ÜBER DIE RADIOAKTIVEN SUBSTANZEN. Von MME. CURIE. Uebersetzt und mit Literatur-Ergänzungen versehen von W. KAUFMANN. Mit eingedruckten Abbildungen. Braunschweig: Druck und Verlag von Friedrich Vieweg und Sohn. 8vo. 1904. pp. viii + 132. Price, 3 marks.

The publishers of this little volume announce that they propose to issue from time to time similar monographs on various subjects dealing with the natural sciences and mathematics. The treatment is not to be popular in the ordinary sense of the word, but the monographs will be intended for specialists and will therefore give some of the experimental and theoretical methods that have been adopted by investigators. This volume will be welcomed by physicists and chemists the world over. It brings together in clear form the principal results of the investigations upon the subject of radio-active substances which have hitherto been published. It is essentially the dissertation of Mme. Curie. The author has, however, not confined herself wholly to her own work, but has embodied all important investigations that have been carried out up to the latter part of 1903. Every one interested in the subject of radio-activity should have a copy of this book. It need scarcely be said that the treatment is clear and interesting. I. R.

PHYSICAL CHEMISTRY IN THE SERVICE OF THE SCIENCES. By JACOBUS H. VAN 'T HOFF, Member of the Prussian Academy of Sciences, Professor Ordinarius, Honorarius in the University of Berlin. English version by ALEXANDER SMITH, of the Department of Chemistry, University of Chicago. University of Chicago Press. 1903.

As is well known, Prof. van't Hoff came to this country and gave a brief course of lectures at the University of Chicago at

[1] This JOURNAL, 31, 268.

their decennial celebration. The present little volume contains an account of what he said. The first, or introductory lecture, discusses the rise of the new Physical Chemistry. The second and third lectures deal with the relations between Physical Chemistry and Pure Chemistry, and for the chemist are the most interesting part of the course. The great contributions of physical chemistry to chemistry in general are through the extension of Avogadro's law to solutions, through the applications of thermodynamics and through the theory of electrolytic dissociation.

Two lectures were devoted to the applications of Physical Chemistry to Industrial Chemistry, two to the relations between Physical Chemistry and Physiology, and two to the bearing of Physical Chemistry on Geology.

The lectures present in a clear and concise form some of the more important relations between physical chemistry and other branches of natural science, and will prove interesting reading to the chemist, physiologist, and geologist.

H. C. J.

A Short Hand-Book of Oil Analyses. By Augustus H. Gill, S.B., Ph.D. Third Edition, Revised and Enlarged. Philadelphia and London: J. B. Lippincott Company. 1903. pp. 158.

Dr. Gill has thoroughly revised the former editions and by the addition of a number of new methods brought the work up to date. The book is divided into two parts. In Part I., Physical and Chemical Tests, the author has included only the standard methods, and while he has described these in a most concise manner he has not omitted to call attention to their weaknesses and limits as to accuracy.

The petroleum products for lubricating and burning purposes are considered first and then the vegetable and animal oils, concluding with a short chapter on the method of examination of an unknown oil.

In Part II., Derivation, Description, and Examination of Certain Oils, the author has given in a very brief way the more important facts about the common oils as to their source, use, and probable adulterants.

The petroleum products are taken up first, giving a list of the various distillates and fractions with their commercial names, boiling-points, and specific gravities. Then the vegetable and animal oils are considered. The ordinary constants are given with a short discussion of their value in detection of adulteration.

In the appendix there are a number of valuable tables on flashing-points of oils and the various state and city regula-

tions. Comparison of viscosimeters, constants of various oils and fatty acids, and comparison of iodine numbers by the Hübl, Hanus, and Wijs methods are given, and also a list of reagents and how to prepare them. In closing, the specifications are given for oils used by the Pennsylvania Railroad.

In the description of the Hanus method for iodine absorption, the addition of 100 cc. of water before the titration has been omitted.

In a number of places the author has omitted to state whether Fahrenheit or Centigrade degrees are meant. In some cases there would be doubt in the mind of the student.

The Bömer method for the separation of phytosterol and cholesterol as given on page 86 is quite different from that given in his original article *Ztschr. Nahr. u. Genus.*, **1**, 38 (1898), and would hardly be satisfactory if used as directed.

These are, however, unimportant points. The author has certainly succeeded in collecting a very large amount of accurate information in a very small space, and the book, while especially fitted for the needs of the student of technical chemistry, is very valuable as a source of ready reference in the laboratory of the oil analyst. L. M. T.

FRACTIONAL DISTILLATION. By SYDNEY YOUNG, D.Sc., F.R.S., Professor of Chemistry in University College, Bristol. With 72 illustrations. London: Macmillan & Co., Limited; New York: The Macmillan Company. 8vo. 1903. pp. xii + 284.

Having been engaged for a number of years on investigations in which it was necessary to use substances in a state of utmost purity, the author naturally resorted frequently to fractional distillation as a means of purification. This led to a familiarity with the methods and forms of apparatus devised by others, while new means were also invented to overcome special difficulties. "It is in the hope that the solution of the difficulties which so often occur in carrying out a fractional distillation may be rendered easier, and that the value and economy of highly efficient still-heads in laboratory work may come to be more widely recognized than is generally the case at present, that this book is written." The whole subject is treated quite fully, having chapters on the apparatus used, including thermometer and barometer corrections, vapor pressures and boiling-points of mixed liquids, modifications of the still-head, mixtures of constant boiling-point, etc. The subject is treated both from the experimental and theoretical points of view, and the results and formulas of other workers are given, the references being placed at the end of each chapter. The chapters that are of most importance to chemists

generally are the three that discuss the modifications of the still-head. "Of all possible forms the plain vertical still-head is the least efficient," yet it is the one most frequently used in laboratories. Even it may have its efficiency increased by being made longer and narrower, although the limit is soon reached on account of the tendency of the condensed liquid in it to be hurled upward by the rising vapors. The simplest improvement is to slant the still-head. Not only is the efficiency increased, but the amount of liquid lost by adhering to the walls is decreased. A spiral still-head carries the idea, and also the efficiency a step further, and the amount of loss is even less, for a given length of tube. In general, it may be said that any of the well-known modifications of the still-head has greater efficiency than the form in common use. The one which, on the whole, is preferable, causes the ascending vapors to pass through the condensed liquid in the stem most frequently, and at the same time is the one in which least liquid remains at the end of the operation. The book is one that will repay careful reading on account of the many valuable suggestions contained in its pages. C. E. W.

TRAITÉ D'ANALYSE DES SUBSTANCES MINÉRALES. Par ADOLPHE CARNOT, Membre de l'Institut, Directeur de l'École Supérieure des Mines. Tome second: Metalloïdes. Ch. Dunod, Éditeur, Paris. Grand in-8 de 821 pages, avec figures. 25 fr.

The first volume of this work explained the different methods in use for the qualitative examination and the quantitative analysis of mineral substances. The second volume is devoted to the analytical study of the metalloids, while the metals are to be treated in the two succeeding volumes. Among the metalloids are included not only those elements usually so classified, but also a number of rarer elements which are of a metallic nature, but whose properties and compounds strongly resemble those of undoubted non-metals. Among these are tungsten, vanadium, molybdenum, titanium, etc. In justification of this course it is pointed out that these elements modify the properties of iron, in particular, in much the same way as carbon, silicon, phosphorus, sulphur, etc.

Each element is taken up in a separate chapter, in which its occurrence, its properties and those of its principal compounds, and the chief reactions having an analytical bearing are given fully. The methods of separation and analysis are discussed in detail. Many of the subjects dealt with in this volume are of especial interest in industrial chemistry, agriculture, and hygiene. For instance, ammonia, nitric and nitrous acids, the hydrocarbons, mineral fuels, the cyanides, the halogens,

including bleaching agents, sulphides and sulphuric acid, phosphates, arsenic compounds, borates, natural and commercial silicates, the minerals and compounds of titanium, tungsten, molybdenum, etc. The book is one that must prove of value in chemical libraries. C. E. W.

KARL HEUMANN'S ANLEITUNG ZUM EXPERIMENTIEREN BEI VORLESUNGEN ÜBER ANORGANISCHEN CHEMIE, zum Gebrauch an Universitäten, technischen Hochschulen und höheren Lehranstalten. Von PROF. DR. O. KÜHLING. Dritte Auflage. Mit 404 in den Text eingedruckten Abbildungen. Braunschweig: Druck and Verlag von Friedrich Vieweg und Sohn. 1904. xxix + 818 Seiten.

The present edition of this well-known work on lecture experiments is not simply a reprint of the preceding edition, although nearly ten years have elapsed since the death of the original author. This could not be the case if the book was to be up to date, on account of the enormous development of inorganic chemistry along different lines, especially physical chemistry, electrochemistry, and numerous investigations at high and low temperatures. Most of the new experiments described illustrate the principles of electrochemistry, although the use of liquid air, the electric furnace, and the Goldschmidt process (indexed as "Aluminothermie") is not overlooked. The character of most physical-chemical experiments is such as to make them unsuitable for lecture demonstrations. The number would be still smaller if the lantern could not be used. This instrument has become so useful that the necessary electrical equipment for it, as well as for the furnace, receive considerable attention in this edition. Most teachers are satisfied with the books of Newth or Benedict, which contain more than enough material for the average course of lectures, but it is well to have at hand some larger work as a reference book. For this purpose there is probably none better than "Heumann." C. E. W.